ÖGH Herpetozoa
Austrian Herpetological Society

Herpetozoa 38: 117–136 (2025)
DOI 10.3897/herpetozoa.38.e155430

A new species of the genus *Boulenophrys* (Anura, Megophryidae) from Guizhou, China

Jing Liu[1,2*], Chao-Bo Feng[2*], Tuo Shen[2], Shi-Ze Li[1,2,3], Yanlin Cheng[1], Gang Wei[4], Bin Wang[3], Haijun Su[2]

1 *Department of Resources and Environment, Moutai Institute, Renhuai 564500, China*
2 *College of Forestry, Guizhou University, Guiyang, 550002, China*
3 *CAS Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization & Ecological Restoration Biodiversity Conservation Key Laboratory of Sichuan Province, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China*
4 *Biodiversity Conservation Key Laboratory, Guiyang College, Guiyang, 550002, China*

https://zoobank.org/9FDADB27-63C0-41C2-9A42-2E19DDB20F55

Corresponding authors: Haijun Su (hjsu@gzu.edu.cn); Bin Wang (wangbin@cib.ac.cn)

Academic editor: Günter Gollmann ♦ **Received** 10 April 2025 ♦ **Accepted** 25 May 2025 ♦ **Published** 13 June 2025

Abstract

Based on morphological and molecular phylogenetic analyses, a new species of the genus *Boulenophrys* is described from Yezhong Nature Reserve, Shuicheng County, Guizhou Province, China. Phylogenetic analyses based on the mitochondrial genes 16S rRNA and COI indicate that this new species represents an independent species-level lineage, closely related to *B. fanjingmontis*, *B. qianbeiensis*, *B. sangzhiensis*, and *B. spinata*. The new species is distinguished from its congeners by a combination of the following characteristics: (1) body size moderate (SVL 41.2–46.2 mm in adult males and 51.8–58.6 mm in adult females); (2) a single small horn-like tubercle at the edge of each upper eyelid; (3) vomerine teeth absent; (4) tongue not notched posteriorly; (5) tympanum distinctly visible, round; (6) toes with rudimentary webbing and narrow lateral fringes; (7) heels overlapping when thighs are positioned at right angles to the body; (8) tibiotarsal articulation reaching the level of the middle of the eye when the leg is stretched forward; (9) a single internal subgular vocal sac in males; (10) in breeding males, the nuptial pads have black nuptial spines on the bases of the first and second fingers.

Key Words

Boulenophrys yezhongensis sp. nov., molecular phylogenetic analysis, morphology, taxonomy

Introduction

The genus *Boulenophrys* Fei, Ye & Jiang, 2016 comprises 71 recognized species, which are classified within the subfamily Megophryinae (Bonaparte 1850) and are widely distributed in eastern and central China, throughout southeastern Asia, and extending to the islands of the Sunda Shelf and the Philippines, westward to Nepal and northeastern India (Frost 2025). The taxonomic arrangements, especially regarding generic assignments within the group, have been controversial for a long time (e.g., Tian and Hu 1983; Dubois 1987; Rao and Yang 1997; Lathrop 1997; Delorme et al. 2006; Jiang et al. 2008; Fei et al. 2009; Fei and Ye 2016; Chen et al. 2017; Mahony et al. 2017; Qi et al. 2021; Lyu et al. 2023). Recently, Lyu et al. (2023) reviewed the literature comprehensively and conducted morphological and molecular phylogenetic analyses, proposing a revised generic classification for Megophryinae. This classification divides the subfamily into ten genera.

* These authors have contributed equally to this work.

Copyright *Jing Liu et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Among these genera, *Boulenophrys* Fei, Ye and Jiang 2016 is one of the most widely distributed and speciose taxa, and 47 species of this genus have been described in the last decade (Frost 2025). Obviously, the diversity of this genus has been underestimated, and new species continue to be discovered and described (Zeng et al. 2024; Xiao et al. 2025; Frost 2025).

During a recent amphibian survey in the Yezhong Nature Reserve, Guizhou Province, China, eight specimens of Asian horned toads were collected. After molecular phylogenetic analyses and morphological comparisons, the results indicated that the specimens from Yezhong Nature Reserve represent an undescribed taxon within the genus *Boulenophrys*. Herein, we describe it as a new species.

Materials and methods

Sampling

Five adult males and three adult females of the undescribed species were collected in Yezhong Nature Reserve (26.1638°N, 104.8803°E, 2022 m), Shuicheng County, Guizhou Province, China (Table 1; Fig. 1). In the field, the toads were euthanized by means of isoflurane (Herrel 2001), and the specimens were fixed in 10% buffered formalin and later transferred to 75% ethanol for preservation. The muscle samples used for molecular analysis were preserved in 95% alcohol and stored at −20 °C. The specimens were deposited in the Chengdu Institute of Biology, Chinese Academy of Sciences (**CIB, CAS**).

Molecular data and phylogenetic analyses

We obtained sequences from eight specimens of the undescribed species. Three specimens of *B. fanjingmontis* were sequenced and included in the molecular analyses (Table 1). Total DNA was extracted using a standard phenol-chloroform extraction protocol (Sambrook et al. 1989). Two fragments of the mitochondrial 16S rRNA (16S) and cytochrome oxidase subunit I (COI) genes were amplified. For 16S, the primers P7 (5'-CGCCTGTTTACCAAAAACAT-3')

Table 1. Samples used in molecular phylogenetic analyses in this study.

ID	*Boulenophrys* species	Voucher ID	Locality	16S rRNA	CO1
1	*B. yezhongensis* sp. nov.	CIBSC20240531001	China,Guizhou,Shuicheng	PV469438	PV491548
2	*B. yezhongensis* sp. nov.	CIBSC20240531002	China,Guizhou,Shuicheng	PV469439	PV491549
3	*B. yezhongensis* sp. nov.	CIBSC20240531004	China,Guizhou,Shuicheng	PV469440	PV491550
4	*B. yezhongensis* sp. nov.	CIBSC20240531005	China,Guizhou,Shuicheng	PV469441	PV491551
5	*B. yezhongensis* sp. nov.	CIBSC20240531003	China,Guizhou,Shuicheng	PV469442	PV491552
6	*B. yezhongensis* sp. nov.	CIBSC20240531008	China,Guizhou,Shuicheng	PV469443	PV491553
7	*B. yezhongensis* sp. nov.	CIBSC20240531006	China,Guizhou,Shuicheng	PV469444	PV491554
8	*B. yezhongensis* sp. nov.	CIBSC20240531007	China,Guizhou,Shuicheng	PV469445	PV491555
9	*B. acuta*	SYS a002266	China, Guangdong, Fengkai	KJ579119	MH406122
10	*B. angka*	KIZ 040591	Thailand, Chiang Mai, Doi Inthanon	MN508052	/
11	*B. anlongensis*	CIB AL20190531018	China, Guizhou, Anlong	MT823184	MT823261
12	*B. baishanzuensis*	CIB QY20200719002	China, Zhejiang, Qingyuan	MW001151	MT998292
13	*B. baolongensis*	KIZ 019216	China, Chongqing, Wushan	KX811813	KX812093
14	*B. binchuanensis*	KIZ 019441	China, Yunnan, Mt Jizu	KX811849	KX812112
15	*B. binlingensis*	KIZ 025807	China, Sichuan, Mt Wawu	KX811852	KX812115
16	*B. binlingensis*	SYSa005313	Wawu Shan, Sichuan, China	MH406892	MH406354
17	*B. binlingensis*	SYSa005314	Wawu Shan, Sichuan, China	MH406893	MH406355
18	*B. boettgeri*	KIZ YPXJK033	China, Fujian, Mt Wuyi	KX811814	KX812104
19	*B. brachykolos*	SYS a002258	China, Hong Kong	KJ560403	MH406120
20	*B. caobangensis*	IEBR 4385	Vietnam, Cao Bang, Nguyen Binh	LC483945	/
21	*B. caudoprocta*	SYS a004293	China, Hunan, Sangzhi	MH406796	MH406258
22	*B. cheni*	SYS a004050	China, Jiangxi, Mt Jinggang	MF667873	MH406241
23	*B. chishuiensis*	SYS a004953	China, Guizhou, Chishui	MH406867	MH406329
24	*B. congjiangensis*	GZNU 20200706003	China, Guizhou, Congjiang	MW959773	MW959761
25	*B. daiyunensis*	SYS a007711	China, Fujian, Tong'an	MW367054	MW365504
26	*B. daoji*	SYS a006212	China, Zhejiang, Mt Tiantai	MW367047	MW365497
27	*B. daweimontis*	KIZ 048997	China, Yunnan, Mt Dawei	KX811867	KX812125
28	*B. dongguanensis*	SYS a002007	China, Guangdong, Dongguan	MH406654	MH406090
29	*B. dupanglingensis*	HUNU 22SA05	China,Hunan,Dupangling Nature Reserve	OP548599	OP550328
30	*B. elongata*	GEPa155	China, Guangdong, Huizhou, Mt Lianhua	OR601595	OR597101
31	*B. fanjingmontis*	SYS a004350	China, Guizhou, Mt Fanjing	MH406808	MH406270
32	*B. fanjingmontis*	FJS20190728001	China, Guizhou, Mt Fanjing	PV469447	PV485343
33	*B. fanjingmontis*	FJS20190728002	China, Guizhou, Mt Fanjing	PV469448	PV485344
34	*B. fanjingmontis*	FJS20190728003	China, Guizhou, Mt Fanjing	PV469449	PV485345
35	*B. fansipanensis*	AMS R186115	Vietnam, Lao Cai, Sapa	MH514887	MW086548

ID	*Boulenophrys* species	Voucher ID	Locality	16S rRNA	CO1
36	*B. fengshunensis*	SYS a004724	China, Guangdong, Fengshun	MH406848	MH406310
37	*B. frigida*	AMS R186131	Vietnam, Lao Cai, Bat Xat	MT364279	MW086550
38	*B. gaolanensis*	SYS a009225	China: Guangdong: Zhuhai,Gaolan	PQ217847	PQ218778
39	*B. hengshanensis*	CSUFT HS210612	China, Hunan, Mt Hengshan	ON209291	/
40	*B. hoanglienensis*	VNMN 2018.02	Vietnam, Lao Cai, Sapa	MH514889	MW086551
41	*B. hungtai*	SYS a007577	China, Guangdong, Jiexi	OL635594	OL634861
42	*B. insularis*	SYS a002171	China, Guangdong, Nan'ao	MH406665	MH406105
43	*B. jiangi*	CIB KKS20180722006	China, Guizhou, Kuankuoshui	MN107743	MN107748
44	*B. jingdongensis*	SYS a003928	China, Yunnan, Mt Wuliang	MH406773	MH406232
45	*B. jinggangensis*	SYS a004028	China, Jiangxi, Mt Jinggang	MH406780	MH406239
46	*B. jiulianensis*	SYS a004218	China, Jiangxi, Mt Jiulian	MH406790	MH406252
47	*B. kuatunensis*	SYS a003449	China, Jiangxi, Mt Wuyi	MF667881	MH406206
48	*B. leishanensis*	KIZ 049172	China, Guizhou, Mt Leigong	KX811825	KX812102
49	*B. liboensis*	GNUG 20150813001	China, Guizhou, Libo	MF285253	/
50	*B. lichun*	CIB 121428	China, Fujian, Ningde	PQ309137	PQ300665
51	*B. lini*	KIZ 07053	China, Jiangxi, Mt Jinggang	KX811842	KX812110
52	*B. lishuiensis*	SYS a008445	China, Zhejiang, Liandu	OQ180984	OQ180872
53	*B. lushuiensis*	CIB YN201909289	China, Yunnan, Lushui	MW001226	MW000913
54	*B. minor*	KIZ YPX37545	China, Sichuan, Dujiangyan	KX811895	KX812144
55	*B. mirabilis*	SYS a002289	China, Guangxi, Lingui	MH406681	MH406127
56	*B. mufumontana*	SYS a006419	China, Hunan, Mt Mufu	MK524107	MK524138
57	*B. nankunensis*	SYS a004503	China, Guangdong, Mt Nankun	MH406824	MH406286
58	*B. nanlingensis*	SYS a001962	China, Guangdong, Ruyuan	MH406645	MH406081
59	*B. obesa*	SYS a002275	China, Guangdong, Fengkai	KJ579123	MH406125
60	*B. ombrophila*	CIB WY18082308	China, Fujian, Mt Wuyi	MW001159	MT998300
61	*B. omeimontis*	KIZ 025765	China, Sichuan, Mt Emei	KX811884	KX812136
62	*B. palpebralespinosa*	KIZ 011650	Vietnam, Thanh Hoa, Pu Hu	KX811889	KX812138
63	*B. pepe*	GEP a207	China, Guangdong, Qingyuan	PQ131151	PQ130479
64	*B. puningensis*	SYS a005770	China, Guangdong, Puning	OL635585	OL634853
65	*B. qianbeiensis*	CIBTZ20190608015	China,Guizhou,Huanglian Nature Reserve	MT651553	MT654520
66	*B. qianbeiensis*	CIBTZ20190608017	China,Guizhou,Huanglian Nature Reserve	MT651554	MT654521
67	*B. qianbeiensis*	CIBTZ20160715003	China,Guizhou,Huanglian Nature Reserve	MT651555	MT654522
68	*B. qianbeiensis*	CIBKKS20180722002	China,Guizhou,Huanglian Nature Reserve	MT651556	MT654523
69	*B. qianbeiensis*	CIBKKS20180722001	China,Guizhou,Huanglian Nature Reserve	MT651557	MT654524
70	*B. rubrimera*	AMS R177676	Vietnam, Lao Cai, Sapa	MF536419	MW086542
71	*B. sangzhiensis*	SYS a004313	China, Hunan, Sangzhi	MH406802	MH406264
72	*B. sangzhiensis*	CIBSZ2012062005	China,Hunan,Badagongshan Nature Reserve	MT651558	MT654525
73	*B. sangzhiensis*	CIBSZ2012062008	China,Hunan,Badagongshan Nature Reserve	MT651559	MT654526
74	*B. sangzhiensis*	CIBSZ2012062007	China,Hunan,Badagongshan Nature Reserve	MH406798	MH406260
75	*B. sanmingensis*	SYS a007057	China, Jiangxi, Mt Magu	MW367051	MW365501
76	*B. shimentaina*	SYS a002077	China, Guangdong, Yingde	MH406655	MH406092
77	*B. shuichengensis*	CIBDF202100812001	China,Guizhou,Dafang	OK017489	OK012066
78	*B. shunhuangensis*	HNNU 16SH04	China, Hunan, Mt Shunhuang	MK836027	/
79	*B. spinata*	KIZ 016100	China, Guizhou, Mt Leigong	KX811864	KX812119
80	*B. spinata*	CIBLS20190801002	China,Guizhou,Mt Leigong	MT651551	MT654518
81	*B. spinata*	CIBLS20190801001	China,Guizhou,Mt Leigong	MT651552	MT654519
82	*B. spinata*	ZL-2018	China,Guizhou,Mt Leigong	MH406676	MH406116
83	*B. tongboensis*	SYS a003227	China, Jiangxi, Mt Tongbo	MH406744	MH406201
84	*B. tuberogranulata*	KIZ YPX10987	China, Hunan, Sangzhi	KX811823	KX812095
85	*B. wugongensis*	SYS a004801	China, Jiangxi, Anfu	MH406854	MH406316
86	*B. wuliangshanensis*	SYS a003924	China, Yunnan, Mt Wuliang	MH406771	MH406230
87	*B. wushanensis*	KIZ YPX47799	China, Chongqing, Wushan	KX811835	/
88	*B. xiangnanensis*	SYS a002874	China, Hunan, Shuangpai	MH406713	MH406165
89	*B. xianjuensis*	CIB XJ190503	China, Zhejiang, Xianju	MN563758	MN563774
90	*B. xuefengmontis*	SYS a007227	China, Hunan, Mt Xuefeng	OQ180973	OQ180861
91	*B. yangmingensis*	SYS a002888	China, Hunan, Shuangpai	MH406719	MH406171
92	*B. yaoshanensis*	SYS a004878	China, Guangxi, Jinxiu	MH406863	MH406325
93	*B. yingdeensis*	SYS a004721	China, Guangdong, Yingde	MH406846	MH406308
94	*B. yunkaiensis*	SYS a004694	China, Guangdong, Xinyi	MH406845	MH406307
95	*Xenophrys mangshanensis*	SYS a002177	China, Guangdong, Huaiji	MH406666	MH406106
96	*Xenophrys glandulosa*	SYS a003757	China, Yunnan, Mt Gaoligong	MH406754	MH406213

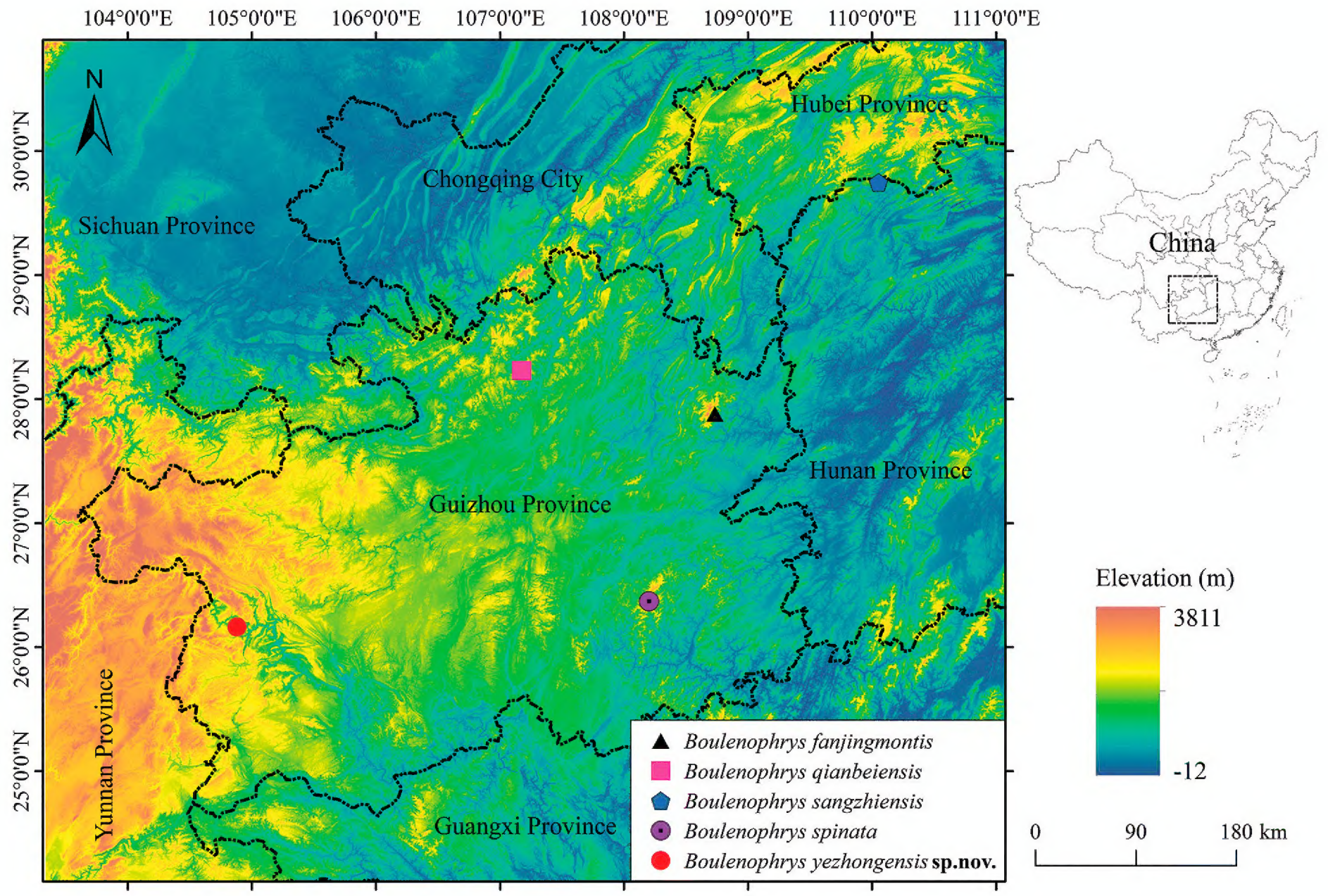

Figure 1. Distribution sites of *Boulenophrys yezhongensis* sp. nov. and its phylogenetically close species, *B. fanjingmontis*, *B. qianbeiensis*, *B. sangzhiensis,* and *B. spinata*.

and P8 (5'-CCGGTCTGAACTCAGATCACGT-3') were used following Simon et al. (1994), and for COI, Chmf4 (5'-TYTCWACWAAYCAYAAAGAYATCGG-3') and Chmr4 (5'-ACYTCRGGRTGRCCRAARAATCA-3') were used following Che et al. (2012). Gene fragments were amplified under the following conditions: an initial denaturation step at 95 °C for 4 min; 36 cycles of denaturation at 95 °C for 30 s, annealing at 52 °C (for 16S) / 47 °C (for COI) for 40 s, and extension at 72 °C for 70 s. Sequencing was conducted using an ABI3730 automated DNA sequencer at Shanghai DNA BioTechnologies Co., Ltd. (Shanghai, China). New sequences were deposited in GenBank (for GenBank accession numbers, see Table 1).

For molecular analyses, a total of 96 available sequence data for congeneric species were downloaded from GenBank (Table 1), primarily from previous studies (Liu et al. 2018; Wang et al. 2024; Song et al. 2024; Xiao et al. 2025). For phylogenetic analyses, sequences from *Xenophrys glandulosa* (Fei et al. 1990) and *X. mangshanensis* (Fei et al. 1990) were downloaded (Table 1) and used as outgroups according to Lyu et al. (2023). Sequences were assembled and aligned using the ClustalW module in BioEdit v. 7.0.9.0 (Hall 1999) with default settings. Alignments were checked manually and revised if necessary. Trimming, with gaps partially deleted, was performed using GBLOCKS 0.91b (Castresana 2000). phylogenetic analyses of mitochondrial DNA, the dataset was concatenated with 16S and COI gene sequences. To avoid under- or over-parameterization (Lemmon and Moriarty 2004; McGuire et al. 2007), the best partition scheme and the best evolutionary model for each partition were selected using PARTITIONFINDER v. 1.1.1 (Robert et al. 2012). In this analysis, the 16S gene and each codon position of the COI gene were defined, and the Bayesian Information Criterion was used. The analysis suggested that the best partition scheme is 16S gene/each codon position of the COI gene and selected the GTR + G + I model as the best model for each partition. Phylogenetic analyses were conducted using maximum likelihood (ML) and Bayesian inference (BI) methods, implemented in PhyML v. 3.0 (Guindon et al. 2010) and MrBayes v. 3.12 (Ronquist and Huelsenbeck 2003), respectively. For the ML tree, branch support was estimated from 10,000 nonparametric bootstrap replicates. In BI, two runs, each with four Markov chains, were simultaneously run for 50 million generations with sampling every 1,000 generations. The first 25% of trees were discarded as burn-in, followed by calculations of Bayesian posterior probabilities and a 50% majority-rule consensus of the post-burn-in trees sampled at stationarity. Finally, mean genetic distances between *Boulenophrys* species based on the uncorrected p-distance model were estimated for the COI and 16S genes using MEGA v. 6.06 (Tamura et al. 2013).

Morphological comparisons

A total of 29 specimens, including five males of the new taxon, five males of *B. sangzhiensis*, five males of *B. fanjingmontis*, six males of *B. qianbeiensis*, and five males of *B. spinata*, were measured (Table 1 and Suppl. material 1). Additional morphological data were sourced from the literature (Table 2). The terminology and methods followed Fei et al. (2009). Measurements were taken with a dial caliper to the nearest 0.1 mm. Seventeen morphometric characters of adult specimens were measured:

ED eye diameter (distance from the anterior corner to the posterior corner of the eye);
FL foot length (distance from tarsus to the tip of the fourth toe);
HDL head length (distance from the tip of the snout to the articulation of the jaw);
HDW maximum head width (greatest width between the left and right articulations of the jaw);
HLL hindlimb length (maximum length from the vent to the distal tip of the Toe IV);
IAE distance between posterior corner of eyes;
IFE distance between anterior corner of eyes;
IND internasal distance (minimum distance between the inner margins of the external nares);
IOD interorbital distance (minimum distance between the inner edges of the upper eyelids);
LAL length of lower arm and hand (distance from the elbow to the distal end of the Finger IV);
LW lower arm width (maximum width of the lower arm);
NED nasal to eye distance (distance between the nasal and the anterior corner of the eye);
NSD nasal to snout distance (distance between the nasal and the posterior edge of the vent);
SVL snout-vent length (distance from the tip of the snout to the posterior edge of the vent);
SL snout length (distance from the tip of the snout to the anterior corner of the eye);
TFL length of foot and tarsus (distance from the tibiotarsal articulation to the distal end of the Toe IV);
THL thigh length (distance from vent to knee);
TL tibia length (distance from knee to tarsus);
TW maximal tibia width;
TYD maximal tympanum diameter;
UEW upper eyelid width (greatest width of the upper eyelid margins measured perpendicular to the anterior-posterior axis).

To reduce the impact of allometry, the ratio of each character to SVL was calculated and log-transformed for subsequent morphometric analyses (Lleonart et al. 2000). One-way analysis of variance (ANOVA) was used to test the significance of differences in morphometric characters between different species. The significance level was set at 0.05. To show the spatial distribution of different species based on morphometric characters, principal component analyses (PCA) were performed. The statistical analyses were conducted using SPSS 21.0 (SPSS Inc., Chicago, IL, USA). The morphology of the new species was also compared with that of all other *Boulenophrys* species (Suppl. material 1, Table 3). Comparative data were obtained from the literature (Table 2).

Results

Phylogenetic analyses

A total of 537 base pairs (bp) of the 16S gene and 558 bp of the COI gene were concatenated into a single 1,095 bp sequence.

ML and BI trees of the mitochondrial DNA dataset presented nearly identical topologies. Although the relationships of many lineages were unresolved (Fig. 2), major nodes in both tree types showed strong support, defined here as Bayesian posterior probabilities (BPP) ≥ 0.95 and maximum likelihood (ML) ultrafast bootstrap values ≥ 95% (Wheeler 2016). In the mitochondrial DNA trees, all samples of the genus *Boulenophrys* in this study clustered within one group, forming a single lineage with high nodal support (BPP = 1.00 and BS = 100%). There was almost no divergence between sequences within this

Table 2. References for morphological characters for congeners of the genus *Boulenophrys*.

ID	Species	References
1	*B. acuta* Wang, Li & Jin, 2014	Li et al. 2014
2	*B. angka* Wu, Suwannapoom, Poyarkov, Pawangkhanant, Xu, Jin, Murphy & Che, 2019	Wu et al. 2019
3	*B. anlongensis*Li, Lu, Liu & Wang, 2020	Li et al. 2020
4	*B. baishanzuensis* Wu, Li, Liu, Wang & Wu, 2020	Wu et al. 2020
5	*B. baolongensis* Ye, Fei & Xie, 2007	Ye et al. 2007
6	*B. binchuanensis* Ye & Fei, 1995	Ye and Fei 1995; Lyu et al. 2023
7	*B. binlingensis* Jiang, Fei & Ye, 2009	Fei et al. 2009; Lyu et al. 2023
8	*B. boettgeri* Boulenger, 1899	Boulenger 1899; Lyu et al. 2023
9	*B. brachykolos* Inger & Romer, 1961	Inger and Romer 1961; Lyu et al. 2023
10	*B. caobangensis* Nguyen, Pham, Nguyen, Luong & Ziegler, 2020	Nguyen et al. 2020
11	*B. caudoprocta* Shen, 1994	Shen. 1994
12	*B. cheni* Wang & Liu, 2014	Wang et al. 2014

ID	Species	References
13	*B. chishuiensis* Xu, Li, Liu, Wei & Wang, 2020	Xu et al. 2020
14	*B. congjiangensis* Luo, Wang, Wang, Lu, Wang, Deng & Zhou, 2021	Luo et al. 2021
15	*B. daiyunensis* Lyu, Wang & Wang, 2021	Lyu et al. 2021
16	*B. daoji* Lyu, Zeng, Wang & Wang, 2021	Lyu et al. 2021
17	*B. daweimontis* Rao & Yang, 1997	Rao and Yang 1997
18	*B. dongguanensis* Wang & Wang, 2019	Wang et al. 2019b
19	*B. dupanglingensis* Xiao & Mo, 2025	Xiao et al. 2025
20	*B. elongata* Zeng, Wang, Chen, Xiao, Zhan, Li & Lin, 2024	Zeng et al. 2024
21	*B. fanjingmontis* Zhang, Liang, Ran & Shen, 2012	Zhang et al. 2012
22	*B. fansipanensis* Tapley, Cutajar, Mahony, Nguyen, Dau, Luong, Le, Nguyen,Nguyen, Portway, Luong & Rowley, 2018	Tapley et al. 2018
23	*B. fengshunensis* Wang, Zeng, Lyu & Wang, 2022	Wang et al. 2022
24	*B. frigida* Tapley, Cutaja, Nguyen, Portway, Mahony, Nguyen, Harding, Luong& Rowley, 2021	Tapley et al. 2021
25	*B. gaolanensis* Song, Wang,Qi, Wang, Wang,2024	Song et al.2024
26	*B. hengshanensis* Qian, Hu, Mo, Gao, Zhang & Yang, 2023	Qian et al. 2023
27	*B. hoanglienensis* Tapley, Cutajar, Mahony, Nguyen, Dau, Luong, Le, Nguyen,Nguyen, Portway, Luong & Rowley, 2018	Tapley et al. 2018
28	*B. hungtai Wang, Zeng, Lyu, Xiao & Wang, 2022*	Wang et al. 2022
29	*B. insularis* Wang, Liu, Lyu, Zeng & Wang, 2017	Wang et al. 2017a
30	*B. jiangi* Liu, Li, Wei, Xu, Cheng, Wang & Wu, 2020	Liu et al. 2020
31	*B. jingdongensis* Fei & Ye, 1983	Fei et al. 1983; Lyu et al. 2023
32	*B. jinggangensis* Wang, 2012	Wang et al. 2012
33	*B. jiulianensis* Wang, Zeng, Lyu & Wang, 2019	Wang et al. 2019b
34	*B. kuatunensis* Pope, 1929	Pope 1929; Lyu et al. 2023
35	*B. leishanensis* Li, Xu, Liu, Jiang, Wei & Wang, 2018	Li et al. 2018
36	*B. liboensis* Zhang, Li, Xiao, Li, Pan, Wang, Zhang & Zhou, 2017	Zhang et al. 2017
37	*B. lichun* Lin, Chen, Li, Peng, Zeng, Wang, 2024	Lin et al.2024
38	*B. lini* Wang & Yang, 2014	Wang et al. 2014
39	*B. lishuiensis* Wang, Liu & Jiang, 2017	Wang et al. 2017b
40	*B. lushuiensis* Shi, Li, Zhu, Jiang, Jiang & Wang, 2021	Shi et al. 2021
41	*B. minor* Stejneger, 1926	Stejneger 1926; Lyu et al. 2023
42	*B. mirabilis* Lyu, Wang & Zhao, 2020	Lyu et al. 2020
43	*B. mufumontana* Wang, Lyu & Wang, 2019	Wang et al. 2019b
44	*B. nankunensis* Wang, Zeng & Wang, 2019	Wang et al. 2019b
45	*B. nanlingensis* Lyu, Wang, Liu & Wang, 2019	Lyu et al. 2019
46	*B. obesa* Wang, Li & Zhao, 2014	Li et al. 2014
47	*B. ombrophila* Messenger & Dahn, 2019	Messenger et al. 2019
48	*B. omeimontis* Liu, 1950	Liu 1950; Lyu et al. 2023
49	*B. palpebralespinosa* Bourret, 1937	Bourret 1937; Lyu et al. 2023
50	*B. pepe* Wang & Zeng, 2024	Wang et al. 2024
51	*B. puningensis* Wang, Zeng, Lyu, Xiao & Wang, 2022	Wang et al. 2022
52	*B. qianbeiensis* Su, Shi, Wu, Li, Yao, Wang & Li, 2020	Su et al. 2020
53	*B. rubrimera* Tapley, Cutajar, Mahony, Chung, Dau, Nguyen, Luong & Rowley,2017	Tapley et al. 2017
54	*B. sangzhiensis* Jiang, Ye & Fei, 2008	Jiang et al. 2008
55	*B. sanmingensis* Lyu & Wang, 2021	Lyu et al. 2021
56	*B. shimentaina* Lyu, Liu & Wang, 2020	Lyu et al. 2020
57	*B. shuichengensis* Tian & Sun, 1995	Tian and Sun 1995
58	*B. shunhuangensis* Wang, Deng, Liu, Wu & Liu, 2019	Wang et al. 2019a
59	*B. spinata* Liu & Hu, 1973	Hu et al. 1973; Lyu et al. 2023
60	*B. tongboensis* Wang & Lyu, 2021	Lyu et al. 2021
61	*B. tuberogranulatus* Shen, Mo & Li, 2010	Mo et al. 2010
62	*B. wugongensis* Wang, Lyu & Wang, 2019	Lyu et al. 2019b
63	*B. wuliangshanensis* Ye & Fei, 1995	Ye and Fei 1995
64	*B. wushanensis* Ye & Fei, 1995	Ye and Fei 1995
65	*B. xiangnanensis* Lyu, Zeng & Wang, 2020	Lyu et al. 2020
66	*B. xianjuensis* Wang, Wu, Peng, Shi, Lu & Wu, 2020	Wang et al. 2020
67	*B. xuefengmontis* Lyu & Wang, 2023	Lyu et al. 2023
68	*B. yangmingensis* Lyu, Zeng & Wang, 2020	Lyu et al. 2020
69	*B. yaoshanensis* Qi, Mo, Lyu, Wang & Wang, 2021	Qi et al. 2021
70	*B. yingdeensis* Qi, Lyu, Wang & Wang, 2021	Qi et al. 2021
71	*B. yunkaiensis* Qi, Wang, Lyu & Wang, 2021	Qi et al. 2021

Table 3. Morphometric comparisons between *Boulenophrys yezhongensis* sp. nov., *B. fanjingmontis*, *B. qianbeiensis*, *B. sangzhiensis*, and *B. spinata*. Units given in mm. Abbreviations for the species name: BF, *B. fanjingmontis*; BQ, *B. qianbeiensis*; BSZ, *B. sangzhiensis*; BSP, *B. spinata*. See abbreviations for morphometric characters in the Materials and Methods section. Significant level at 0.05.

Measurements	*B.yezhongensis* sp. nov				*B. fanjingmontis*		*B. qianbeiensis*		*B. sangzhiensis*		*B. spinata*		*p*-value from ANOVA in male			
	males (n = 5)		females (n = 3)		males (n = 5)		males (n = 6)		males (n = 5)		males (n = 5)					
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	sp vs. BSZ	sp vs. BQ	sp vs. BS	sp vs. BF
SVL	41.2–46.2	43.5 ± 1.9	51.8–58.6	54.1 ± 3.9	58.2–67.7	63.2 ± 4.0	49.3–58.2	54.3 ± 3.1	56.1–59.8	58.3 ± 1.6	51.2–56.2	53.9 ± 1.8	0.00	0.00	0.00	0.00
HDL	12.1–13.0	12.6 ± 0.3	14.2–15.2	14.6 ± 0.6	16.1–18.0	17.1 ± 0.8	14.6–17.0	15.6 ± 0.9	16.1–18.0	17.2 ± 1.0	14.3–15.8	14.8 ± 0.6	0.57	0.99	0.10	0.09
HDW	14.3–15.4	14.8 ± 0.4	16.9–17.4	17.2 ± 0.3	20.3–22.9	21.9 ± 1.1	18.3–21.0	19.9 ± 1.1	20.0–21.5	20.8 ± 0.6	18.4–19.1	18.7 ± 0.3	0.03	0.01	0.48	0.38
SL	5.2–5.8	5.5 ± 0.3	6.0–7.1	6.5 ± 0.5	6.7–8.1	7.5 ± 0.5	6.7–7.2	6.9 ± 0.2	6.6–8.2	7.4 ± 0.6	5.7–7.1	6.4 ± 0.6	0.81	0.94	0.20	0.16
TYD	2.8–3.3	3.0 ± 0.2	3.0–3.7	3.4 ± 0.3	3.1–3.6	3.4 ± 0.2	3.2–4.3	3.5 ± 0.4	3.1–3.7	3.4 ± 0.2	2.5–2.9	2.7 ± 0.2	0.01	0.42	0.00	0.00
IFE	6.7–7.5	7.2 ± 0.3	7.5–7.9	7.7 ± 0.2	10.7–11.7	11.0 ± 0.5	7.8–10.9	9.7 ± 1.1	9.4–10.9	10.2 ± 0.5	8.5–9.6	9.1 ± 0.4	0.02	0.10	0.41	0.04
IAE	11.6–12.8	12.2 ± 0.5	14.1–14.6	14.4 ± 0.3	16.8–18.0	17.3 ± 0.5	14.0–16.4	15.5 ± 0.9	16.1–17.3	16.8 ± 0.6	14.0–14.4	14.2 ± 0.2	0.33	0.52	0.04	0.48
NED	2.7–2.9	2.8 ± 0.1	2.8–3.1	3.0 ± 0.1	3.5–4.5	4.1 ± 0.4	2.4–3.4	2.9 ± 0.4	3.0–3.9	3.4 ± 0.4	2.7–3.0	2.8 ± 0.1	0.14	0.02	0.00	0.98
NSD	2.4–2.7	2.5 ± 0.1	2.7–3.0	2.8 ± 0.2	3.5–4.1	3.7 ± 0.2	3.5–4.3	3.8 ± 0.3	3.7–4.6	4.2 ± 0.3	3.4–4.1	3.8 ± 0.3	0.00	0.00	0.01	0.81
IND	5.0–5.6	5.3 ± 0.2	5.5–6.0	5.8 ± 0.3	6.5–7.5	7.2 ± 0.4	5.7–7.5	6.7 ± 0.6	6.8–7.7	7.2 ± 0.3	5.8–6.3	6.0 ± 0.2	0.00	0.00	0.02	0.01
IOD	3.9–4.9	4.4 ± 0.4	5.2–5.6	5.3 ± 0.2	5.1–6.1	5.7 ± 0.4	3.7–5.3	4.5 ± 0.6	4.7–5.8	5.1 ± 0.5	4.2–5.3	4.9 ± 0.4	0.00	0.00	0.00	0.00
ED	4.3–5.2	4.9 ± 0.3	5.4–6.2	5.7 ± 0.4	6.3–7.1	6.7 ± 0.3	5.4–6.9	6.3 ± 0.6	6.1–7.4	6.7 ± 0.5	5.1–6.0	5.6 ± 0.3	0.31	0.15	0.18	0.15
UEW	3.8–4.3	4.0 ± 0.2	4.6–5.3	5.0 ± 0.3	5.1–5.9	5.4 ± 0.3	5.1–6.2	5.6 ± 0.4	5.1–6.1	5.7 ± 0.4	4.5–6.1	5.0 ± 0.7	0.10	0.02	0.72	0.10
LAL	18.8–21.3	19.7 ± 1.0	23.3–25.0	23.9 ± 0.9	27.1–29.4	28.7± 1.0	20.4–25.4	24.0 ± 1.8	26.2–28.2	26.9 ± 0.8	24.0–25.1	24.3 ± 0.4	0.39	0.38	0.94	0.69
LW	2.5–3.1	2.9 ± 0.2	2.9–3.3	3.1 ± 0.2	5.5–6.3	5.9 ± 0.4	5.7–7.4	6.6 ± 0.6	6.1–6.7	6.4 ± 0.2	5.5–7.4	6.3 ± 0.7	0.00	0.00	0.00	0.00
HLL	56.5–70.6	63.5 ± 5.2	76.3–84.3	80.1 ± 4.0	100.8–109.3	105.7 ± 3.2	76.9–93.7	87.8 ± 6.1	97.3–105.0	100.3 ± 2.8	85.7–99.0	91.8 ± 5.3	0.00	0.01	0.00	0.00
THL	17.4–20.5	18.6 ± 1.2	21.7–24.4	23.1 ± 1.4	29.7–34.6	32.3 ± 1.8	24.1–28.4	26.6 ± 1.8	27.9–31.6	29.6 ± 1.6	26.1–29.8	27.6 ± 1.4	0.00	0.00	0.00	0.00
TL	19.3–22.4	20.6 ± 1.2	24.6–26.0	25.1 ± 0.8	32.8–35.2	34.1 ± 1.0	25.0–32.4	29.2 ± 2.6	31.6–32.6	32.2 ± 0.4	28.9–30.4	29.6 ± 0.6	0.00	0.00	0.00	0.00
TW	4.2–4.7	4.4 ± 0.2	5.1–5.2	5.2 ± 0.0	6.9–7.8	7.4 ± 0.3	7.0–8.7	8.0 ± 0.6	7.3–8.1	7.7 ± 0.3	6.0–8.0	7.0 ± 0.9	0.00	0.00	0.01	0.00
TFL	26.6–32.4	29.1 ± 2.4	35.1–38.0	36.5 ± 1.5	43.9–47.9	45.7 ± 1.6	34.5–44.0	40.4 ± 3.6	42.9–46.1	44.2 ± 1.3	39.2–43.1	40.9 ± 1.6	0.00	0.02	0.00	0.05
FL	15.7–19.7	17.6 ± 1.5	22.1–23.2	22.6 ± 0.6	29.4–32.5	31.4 ± 1.2	24.5–29.4	27.5 ± 2.9	26.7–29.9	28.9 ± 1.3	26.4–29.6	28.1 ± 1.2	0.00	0.00	0.00	0.00

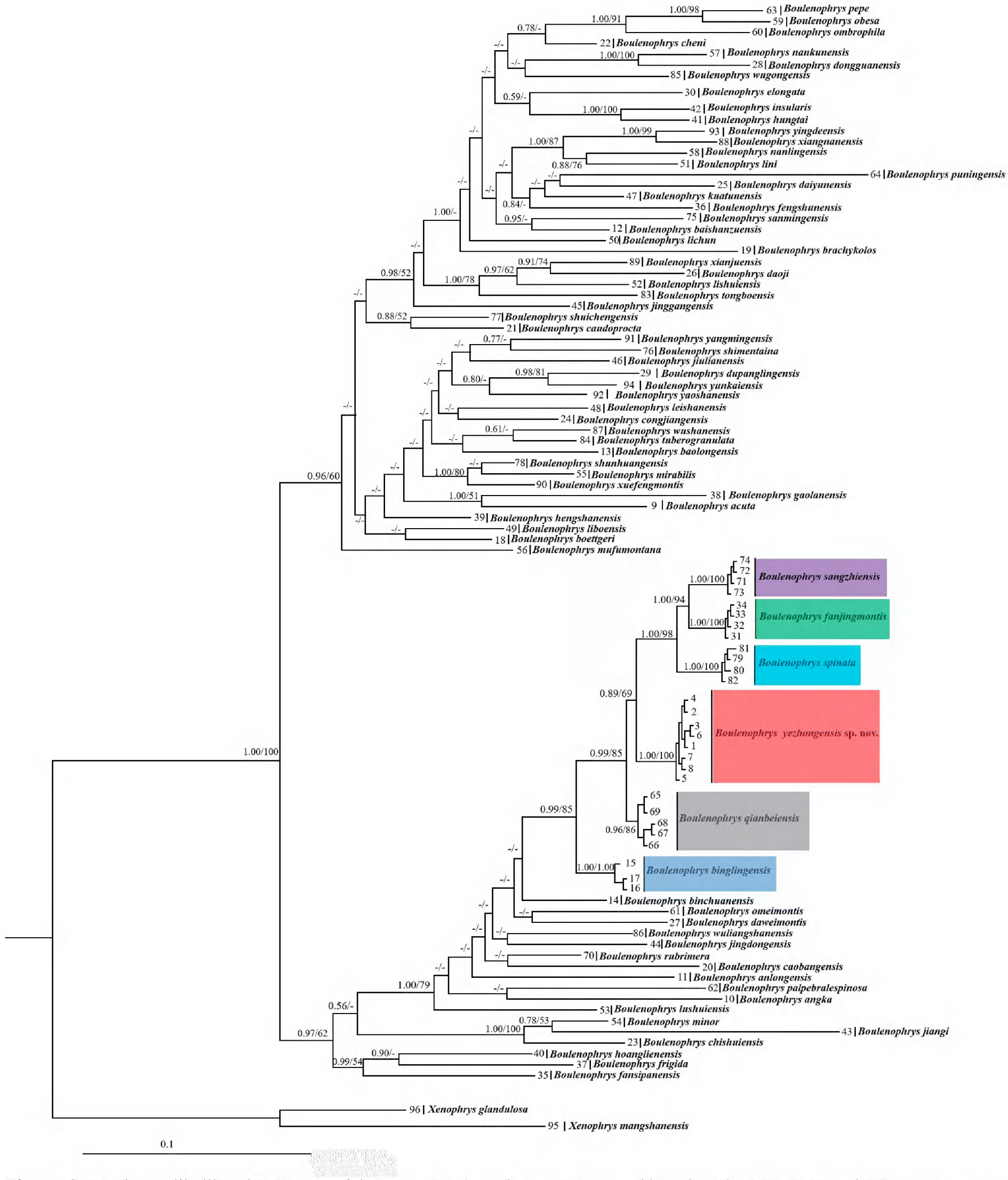

Figure 2. Maximum likelihood (ML) tree of the genus *Boulenophrys* reconstructed based on the 16S rRNA and COI gene sequences. Bayesian posterior probability/ML bootstrap supports were denoted beside each node. Samples 1–96 refer to Table 1.

lineage, and the lineage was sister to a clade composed of *B. fanjingmontis*, *B. qianbeiensis*, *B. sangzhiensis*, and *B. spinata*, with high support.

Genetic distances based on the 16S and COI genes, calculated using the uncorrected p-distance model, showed that divergence within the undescribed species was below 0.1%. The COI gene distance between the undescribed species and its closest related species, *B. qianbeiensis*, was 2.5%; 5.3% with *B. sangzhiensis*; 5.8% with *B. fanjingmontis*; and 5.9% with *B. spinata*. For the 16S gene, the closest genetic distance was 0.6% with *B. binlingensis*, 0.9% with *B. qianbeiensis*, 0.9% with *B. fanjingmontis*, 1.1% with *B. sangzhiensis*, and 1.4% with *B. spinata* (see Suppl. materials 2, 3).

Morphological analyses

The results of the ANOVA tests based on several morphometric characteristics revealed significant differences ($p < 0.05$; Table 3) between male individuals of the new species and males of *B. qianbeiensis*, *B. fanjingmontis*, *B. sangzhiensis*, and *B. spinata*. In the PCA for males, the first two principal components accounted for 61.9% of the total variance (Table 4). Loadings for PC1, which explained 40.5% of the total variance, were most heavily weighted on IND, HLL, TL, TW, TFL, and FL, while loadings for PC2, which explained 21.4%, were most heavily weighted on TYD and IAE. Differentiation was evident along the PC1 axis between the newly collected specimens and *B. qianbeiensis*, *B. sangzhiensis*, *B. spinata*, and *B. fanjingmontis* (Fig. 3). Detailed morphological comparisons revealed discrete diagnostic characters distinguishing the undescribed taxon from its congeners. Based on the molecular phylogenetic analyses and morphological comparisons (see Suppl. material 4), the specimens from Yezhong Nature Reserve represent a new species, which is described as follows.

Table 4. Factor loadings of the first four principal components for 21 size-adjusted male morphometric characteristics of *Boulenophrys yezhongensis* sp. nov., *B. sangzhiensis*, *B.spinata*, *B. qianbeiensis* and *B. fanjingmontis*.

Character	PC1	PC2	PC3	PC4
Eigenvalue	8.497	4.495	2.117	1.274
% variation	40.462	21.406	10.083	6.066
SVL	0.510	-0.606	-0.013	0.417
HDL	0.108	0.751	0.103	-0.155
HDW	0.681	0.613	0.036	0.005
SL	0.103	0.693	-0.005	0.180
NED	-0.403	0.154	0.715	0.127
NSD	0.740	0.274	-0.238	-0.336
IND	**0.820**	0.057	-0.222	0.073
IOD	-0.730	0.350	0.354	-0.301
ED	0.300	0.686	-0.110	0.411
UEW	0.351	0.515	-0.323	-0.205
LAL	0.338	0.194	0.794	-0.063
LW	0.792	-0.099	-0.376	-0.255
HLL	**0.843**	-0.232	0.391	-0.030
THL	0.756	-0.417	0.176	0.062
TL	**0.907**	-0.158	0.260	-0.054
TW	**0.882**	0.211	-0.183	-0.049
TFL1	**0.868**	-0.022	0.318	-0.189
FL	**0.889**	-0.210	0.182	-0.210
TYD	-0.307	**0.806**	-0.104	-0.056
IFE	0.532	0.247	-0.024	0.660
IAE	0.276	**0.804**	0.210	0.100

Taxonomic account

Boulenophrys yezhongensis sp. nov.

https://zoobank.org/34D3EB46-4E41-4EAA-8DC4-066BC038437E

Type material. ***Holotype*** • CIB SC20240531005 (Figs 4, 5), adult male, from Yezhong Nature Reserve, Shuicheng County, Guizhou Province, China (26.163426°N, 104.880083°E, ca. 2022 m a. s. l.), collected by Jing Liu on 31 May 2024.

Paratypes • Seven adult specimens (4 males and 3 females) from the same place as holotype. Four males (CIB SC20240531001– 4) collected on 31 May 2024 by Shize Li and Chaobo Feng. • Three females (CIB SC20240531006–8) collected on 31 May 2024 by Jing Liu and Shize Li.

Diagnosis. *Boulenophrys yezhongensis* sp. nov. is assigned to the genus *Boulenophrys* based on molecular phylogenetic analyses and the following generic diagnostic characters: snout shield-like; projecting beyond the lower jaw; canthus rostralis distinct; chest glands small and round, closer to the axilla than to midventral line; femoral glands on rear part of thigh; vertical pupils; and the presence of subarticular tubercles at the base of each finger (Fei et al. 2016; Lyu et al. 2023).

Boulenophrys yezhongensis sp. nov. can be distinguished from its congeners by a combination of the following morphological characters: body size moderate (SVL 41.2–46.2 mm in adult males and 51.8–58.6 mm in adult females); a small horn-like tubercle at edge of each upper eyelid; vomerine teeth absent; tongue not notched posteriorly; tympanum distinctly visible, round; two metacarpal tubercles on hand; toes with rudimentary webbing and narrow lateral fringes; heels overlapping when thighs are positioned at right angles to the body; tibiotarsal articulation reaching the level to the middle of eye when leg stretched forward; a single internal subgular vocal sac in adult males; in breeding males, nuptial pads with black nuptial spines on the dorsal bases of the first and second fingers.

Description of holotype. CIB SC20240531005 (Figs 4, 5). SVL 44.4 mm; head width greater than head length (HDW/HDL ratio about 1.2); snout obtusely pointed, protruding well beyond the margin of the lower jaw in ventral view; loreal region vertical and concave; canthus rostralis well-developed; top of head flat in dorsal view; eye large, eye diameter 40.0% of head length; pupils vertical; nostril orientated laterally, closer to snout than eye; tympanum distinct, TYP/EYE ratio 0.6; vomerine ridges present and vomerine teeth absent; margin of tongue smooth, not notched behind.

Forelimbs slender and comparatively short relative to those of congeneric species; length of lower arm and hand 44.4% of SVL; fingers robust; relative finger lengths: II < IV< I < III; digit tips globular, without lateral fringes; distinct subarticular tubercles at finger bases; two prominent metacarpal tubercles, oval-shaped, the inner larger than the outer.

Hindlimbs slender (HLL/SVL 1.47); heels overlapping when thighs at right angles to body, tibiotarsal articulation reaching mid-eye when leg stretched forward; tibia longer than thigh; relative toe lengths: I < II < V < III < IV; toes round, slightly dilated; subarticular tubercles at toe base; toes with rudimentary webbing, narrow

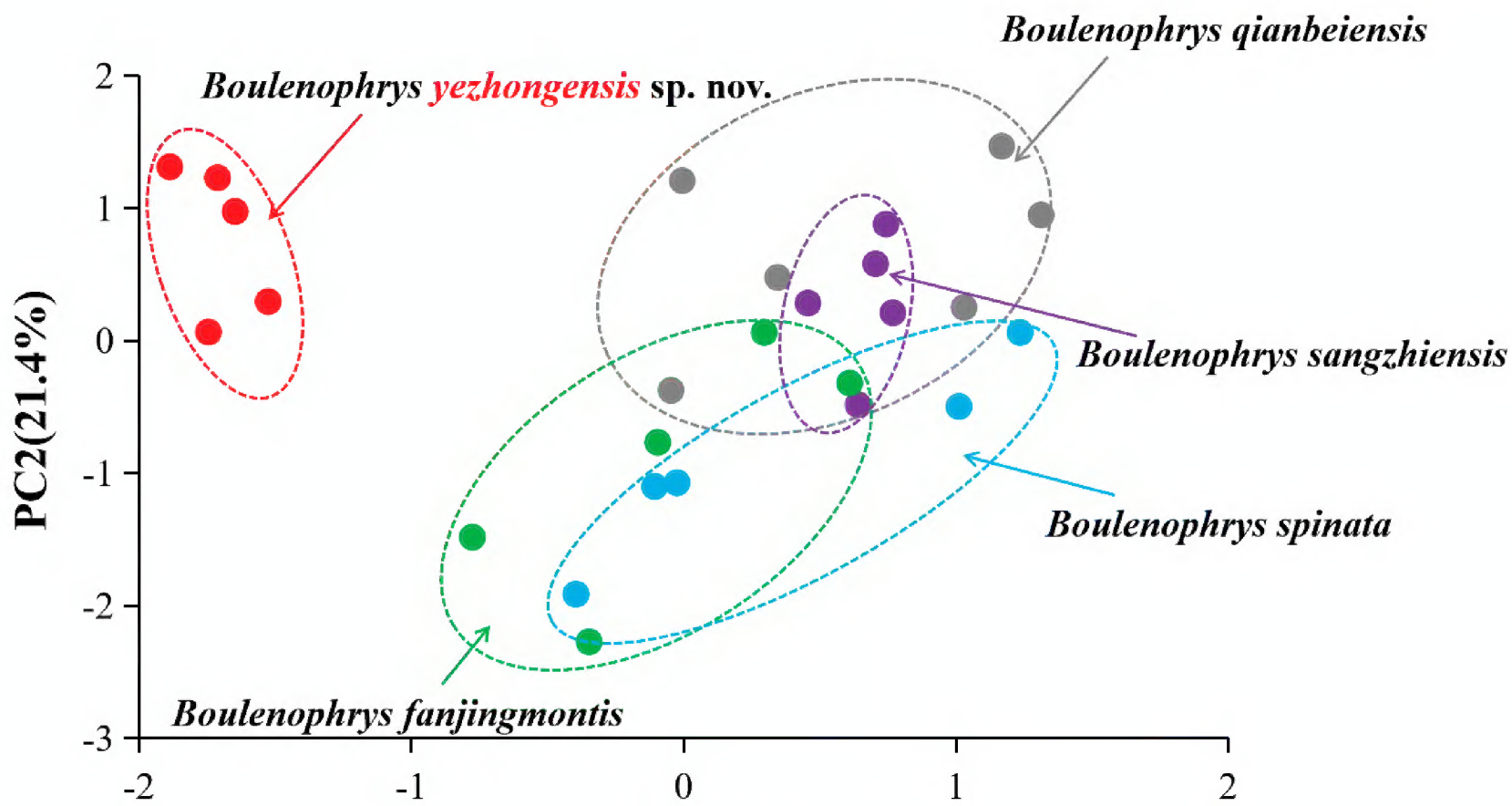

Figure 3. Plots of the first principal component (PC1) versus the second (PC2) for males of *Boulenophrys yezhongensis* sp. nov. and *B. fanjingmontis*, *B. qianbeiensis*, *B. sangzhiensis*, and *B. spinata*.

Figure 4. Male holotype CIB SC20240531005 of *Boulenophrys yezhongensis* sp. nov. in life. **A.** Dorsal view; **B.** Ventral view; **C.** Dorsal view of hand; **D.** Ventral view of hand; **E.** Ventral view of foot.

and barely visible lateral fringe; inner metatarsal tubercle oval-shaped; outer metatarsal tubercle absent.

Dorsal skin rough, with numerous brownish-yellow granules; several large warts scattered on flanks; tubercles on the dorsum forming a weak V-shaped ridge; two discontinuous dorsolateral parallel ridges on either side of the V-shaped ridges; tubercles on flanks, dorsal thighs, and tibias; supratympanic fold distinct; white granules with black hard spines on temporal fold region and adjacent areas.

Ventral surface smooth with numerous white granules; pectoral glands indistinct; femoral glands on rear of thighs; inner thighs and shanks with white granules, each tipped with single black hard spine.

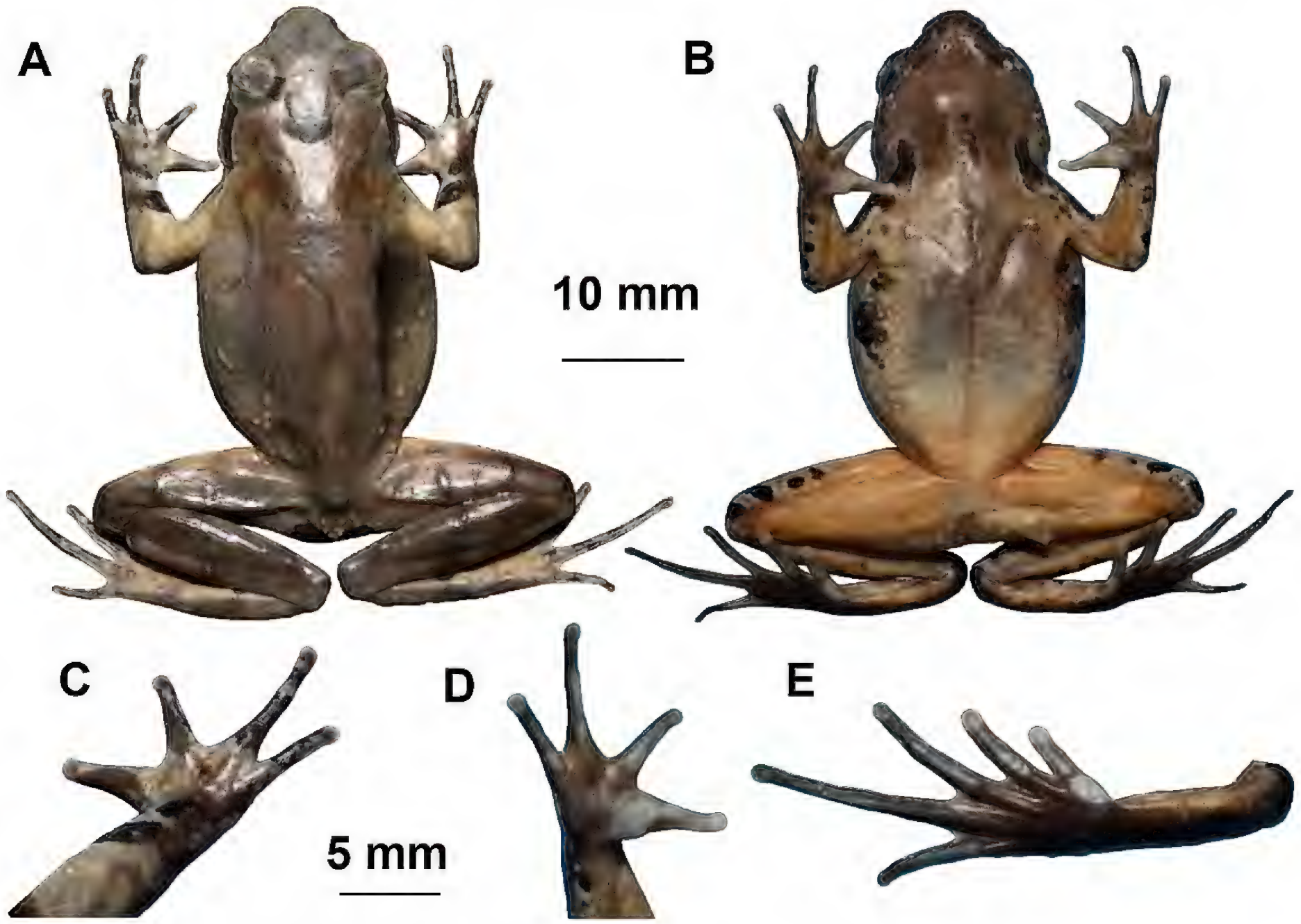

Figure 5. The holotype specimen CIB SC20240531005 of *Boulenophrys yezhongensis* sp. nov. in preservative. **A.** Dorsal view; **B.** Ventral view; **C.** Dorsal view of hand; **D.** Ventral view of hand; **E.** Ventral view of foot.

Coloration of holotype in life. (Fig. 4). Inverted triangular brown speckle between eyes; dorsal V-shaped ridges dotted with brown speckles; dorsal surfaces of the thigh and shank characterized by four prominent dark brown transverse bands; upper and lower lips with alternating dark brown and white vertical bars; belly light orange-red; large white blotch and small grey blotch at center, small white blotches and large black patches on sides (from discontinuous line); posterior limb venters orange, thickly covered with numerous granules; palms, soles, and digit tips purple-grey; femoral glands pure white; iris copper-brown; throat and anterior chest light purple-brown.

Coloration of holotype in preservation. (Fig. 5). Dorsal surface faded to brown; inverted triangular brown speckle between eyes and V-shaped ridges on dorsum indistinct; throat and anterior chest light brownish-tan, abdomen pale orange-red with central white spot, lateral small white speckles interspersed with large black patches; ventral hindlimbs with white tubercles, males with black hardened spines.

Variation. In CIB SC20240531002 (Fig. 6A): Overall color relatively dark; indistinct transverse stripes between thighs and shanks; black spines on white granules sparse, color not prominent. In CIB SC20240531004 (Fig. 6B): Dorsal parallel ridges in reticular pattern. In CIB SC20240531006 (Fig. 6C), tympanic membrane small, color indistinct; dorsal parallel ridges obscure, with only one ridge discernible. In CIB SC20240531008 (Fig. 6D): dorsum dark brownish; markings abundant and broad; wide transverse stripes on thighs and shanks; dorsal wart granules small, several large ones on flanks.

Secondary sexual characters. Adult males (SVL 41.2–46.2 mm) are smaller than adult females (SVL 51.8–58.6 mm). Adult males have a single subgular vocal sac. In breeding males, there are dark black nuptial pads at the bases of the first and second fingers, and these pads are covered with fine, dense black nuptial spines. Moreover, white granules are present on the inner thighs, around the anus, the temporal fold region, and these granules are adorned with black hard spines. In contrast, females possess only white granules.

Comparisons. (Suppl. material 4) *Boulenophrys yezhongensis* sp. nov. is phylogenetically closest to *B. fanjingmontis*, *B. qianbeiensis*, *B. sangzhiensis*, and *B. spinata* (Fig. 7). The new species is easily distinguished from *B. fanjingmontis* by its slightly smaller body size: in adult males, the SVL is 41.2–46.2 mm (vs. 58.2–63.6 mm in the latter), and in adult females, the SVL is 51.8–58.6 mm (vs. 62.8–72.2 mm in the latter); tongue not notched posterior (vs. tongue feebly notched behind in the latter); having narrow lateral fringes on toes (vs. wide lateral fringes in the latter); breeding males have

Figure 6. Color variation in *Boulenophrys yezhongensis* sp. nov. in life. **A.** Dorsolateral view of adult male CIB SC20240531003; **B.** Dorsolateral view of adult male CIB SC20240531002; **C1.** Dorsolateral view of adult female CIB SC20240531006; **C2.** Ventral view of adult female CIB SC20240531006; **D1.** Dorsolateral view of adult female CIB SC20240531007; **D2.** Ventral view of adult female CIB SC20240531007.

dark black nuptial pads at the bases of the first and second fingers, and these pads are covered with fine and dense black nuptial spines (vs. obvious black nuptial spines, spines on nuptial pads are larger and sparser in the latter); the values of morphological characteristics such as SVL, IND, IOD, LW, HLL, THL, TL, TW, TFL, FL, TYD, and IFE are significantly smaller in the new species than those in *B. fanjingmontis* (Table 3).

The new species differs from *B. qianbeiensis* by the following characters: having a slightly smaller body size, in adult males SVL 41.2–46.2 mm (vs. 49.3–58.2 mm in *B. qianbeiensis*), in adult females SVL 51.8–58.6 mm (vs. 65.2 mm in *B. qianbeiensis*); Breeding males have dark black nuptial pads at the bases of the first and second fingers, and these pads are covered with fine and dense black nuptial spines (vs. obvious black nuptial spines, spines on nuptial pads are larger and sparser in the latter); vomerine ridges separated and weak, vomerine teeth absent (vs. vomerine ridges and vomerine teeth present and distinct in the latter); tongue not notched posterior (vs. tongue feebly notched behind in the latter); toes with rudimentary webbing (vs. toes one quarter webbed in the latter); having narrow lateral fringes on toes(vs. wide lateral fringes in the latter); tibiotarsal articulation reaching the middle of eye when leg stretched forward (vs. reaching the level between tympanum and eye in the latter);

Figure 7. Comparison of the morphological characteristics of adult male *Boulenophrys yezhongensis* sp. nov. and its closely related species. **A1–A5.** *Boulenophrys yezhongensis* sp. nov.; **B1–B5.** *B. sangzhiensis*; **C1–C5.** *B. qianbeiensis*; **D1–D5.** *B. spinata*; **E1–E5.** *B. fanjingmontis*.

the values of morphological characteristics such as SVL, HDW, NED, NSD, IND, IOD, UEW, LW, HLL, THL, TL, TW, TFL and FL are significant smaller in the new species than those in *B. qianbeiensis* (Table 3).

The new species differs from *B. sangzhiensis* by the following characters: having a slightly smaller body size, in adult males SVL 41.2–46.2 mm (vs. 55.7–62.2 mm in the latter), in adult females SVL 51.8–58.6 mm (vs. 62.2–76.3 mm in the latter); breeding males have dark black nuptial pads at the bases of the first and second fingers, and these pads are covered with fine and dense black nuptial spines (vs. obvious black nuptial spines, spines on nuptial pads are larger and sparser in the latter); tongue not notched posterior (vs. tongue feebly notched behind in the latter);the values of morphological characteristics such as SVL, HDW, NSD, IND, IOD, LW, HLL, THL, TL, TW, TFL, FL, TYD and IFE are significant smaller in the new species than those in *B. sangzhiensis* (Table 3).

The new species differs from *B. spinata* by the following characters: having a slightly smaller body size, in adult males SVL 41.2–46.2 mm (vs. 47.2–54.4 mm in the latter), in adult females the SVL is not significant different; tongue not notched posterior (vs. tongue feebly notched behind in the latter); toes with rudimentary webbing (vs. toes one quarter webbed in the latter); having narrow lateral fringes on toes(vs. wide lateral fringes in the latter); breeding males have dark black nuptial pads at the base of the first and second fingers, and these pads are covered with fine and dense black nuptial spines (vs. *B. spinata* has obvious black nuptial spines, and the spines on its nuptial pads are larger and sparser); the values of morphological characteristics such as SVL, TYD, IAE, NED, NSD, IND, IOD, LW, HLL, THL, TL, TW, TFL and FL are significant smaller in the new species than those in *B. spinata* (Table 3).

By having medium body size, *Boulenophrys yezhongensis* sp. nov. differs from *B. acuta*, *B. angka*, *B. baishanzuensis*, *B. binchuanensis*, *B. boettgeri*, *B. cheni*, *B. congjiangensis*, *B. daiyunensis*, *B. daoji*, *B. daweimontis*, *B. elongata*, *B. frigida*, *B. gaolanensis*, *B. hungtai*, *B. jinggangensis*, *B. jiulianensis*, *B. kuatunensis*, *B. lichun*, *B. lishuiensis*, *B. lushuiensis*, *B. mufumontana*, *B. nankunensis*, *B. obesa*, *B. ombrophila*, *B. pepe*, *B. puningensis*, *B. rubrimera*, *B. sanmingensis*, *B. shimentaina*, *B. shunhuangensis*, *B. tongboensis*, *B. wugongensis*, *B. wuliangshanensis*, *B. wushanensis*, *B. xianjuensis*, *B. yangmingensis* and *B. yingdeensis*(minimum SVL > 41.0 mm in the new species vs. maximum SVL < 37.0 mm in the latter), and differs from *B. caudoprocta*, *B. fanjingmontis*, *B. jingdongensis*, *B. liboensis*, *B. mirabilis*, *B. omeimontis*, *B. qianbeiensis*, *B. sangzhiensis* and *B. shuichengensis* (maximum SVL < 46.0 mm in the new species vs. minimum SVL > 50.0 mm in the latter).

By vomerine teeth being absent, *Boulenophrys yezhongensis* sp. nov. differs from *B. brachykolos*, *B. caudoprocta*, *B. daiyunensis*, *B. daweimontis*, *B. dongguanensis*, *B. elongata*, *B. fansipanensis*, *B. fengshunensis*, *B. frigida*, *B. hoanglienensis*, *B. insularis*, *B. jingdongensis*, *B. jinggangensis*, *B. jiulianensis*, *B. liboensis*, *B. nankunensis*, *B. nanlingensis*, *B. omeimontis*, *B. palpebralespinosa*, *B. puningensis*, *B. qianbeiensis*, *B. rubrimera*, *B. shimentaina*, *B. tongboensis*, and *B. yingdeensis* (vs. present in the latter).

By the vomerine ridges present, *Boulenophrys yezhongensis* sp. nov. differs from *B. baolongensis*, *B. binchuanensis*, *B. boettgeri*, *B. chishuiensis*, *B. congjiangensis*, *B. daoji*, *B. dupanglingensis*, *B. gaolanensis*, *B. hengshanensis*, *B. hungtai*, *B. leishanensis*, *B. lichun*, *B. lishuiensis*, *B. minor*, *B. mirabilis*, *B. ombrophila*, *B. sanmingensis*, *B. shunhuangensis*, *B. tuberogranulatus*, *B. wuliangshanensis*, *B. wushanensis*, *and B. xuefengmontis* (vs. absent in the latter).

By a small horn-like tubercle present at the edge of each upper eyelid, *Boulenophrys yezhongensis* sp. nov. differs from *B. acuta*, *B. caudoprocta*, *B. jinggangensis*, *B. liboensis*, *B. mirabilis*, and *B. palpebralespinosa* (vs. having a prominent and elongated tubercle in the latter).

By tongue not notched posteriorly, *Boulenophrys yezhongensis* sp. nov. differs from *B. baolongensis*, *B. binlingensis*, *B. boettgeri*, *B. cheni*, *B. fanjingmontis*, *B. insularis*, *B. jingdongensis*, *B. jiulianensis*, *B. kuatunensis*, *B. liboensis*, *B. lushuiensis*, *B. minor*, *B. nanlingensis*, *B. omeimontis*, *B. pepe*, *B. qianbeiensis*, *B. sangzhiensis*, *B. sanmingensis*, *B. shuichengensis*, *B. spinata*, and *B. tongboensis* (vs. tongue feebly notched behind in the latter).

By toes with narrow lateral fringes, *Boulenophrys yezhongensis* sp. nov. differs from *B. angka*, *B. baolongensis*, *B. brachykolos*, *B. caobangensis*, *B. chishuiensis*, *B. daweimontis*, *B. dongguanensis*, *B. dupanglingensis*, *B. fansipanensis*, *B. fengshunensis*, *B. frigida*, *B. gaolanensis*, *B. hengshanensis*, *B. hoanglienensis*, *B. hungtai*, *B. insularis*, *B. jiangi*, *B. jiulianensis*, *B. leishanensis*, *B. lichun*, *B. lishuiensis*, *B. major*, *B. nankunensis*, *B. obesa*, *B. ombrophila*, *B. pepe*, *B. puningensis*, *B. shunhuangensis*, *B. tongboensis*, *B. tuberogranulata*, *B. wugongensis*, *B. wuliangshanensis*, *B. xuefengmontis*, *B. yaoshanensis*, *B. yingdeensis*, and *B. yunkaiensis* (vs. lacking lateral fringes on toes). differs from *B. binchuanensis*, *B. boettgeri*, *B. cheni*, *B. fanjingmontis*, *B. jingdongensis*, *B. liboensis*, *B. lini*, *B. palpebralespinosa*, *B. qianbeiensis*, *B. rubrimera*, *B. sanmingensis*, *B. shuichengensis*, *B. spinata*, and *B. xiangnanensis* (vs. having lateral wide fringes on toes in the latter).

By toes with rudimentary webbing, *Boulenophrys yezhongensis* sp. nov. differs from *B. baishanzuensis*, *B. baolongensis*, *B. daweimontis*, *B. elongata*, *B. fansipanensis*, *B. frigida*, *B. gaolanensis*, *B. hengshanensis*, *B. hungtai*, *B. kuatunensis*, *B. lichun*, *B. lishuiensis*, *B. ombrophila*, *B. pepe*, *B. rubrimera*, *B. shunhuangensis*, *B. tongboensis*, *B. wuliangshanensis*, *and B. xuefengmontis* (vs. toes lacking webbing in the latter) and differs from *B. jingdongensis*, *B. palpebralespinosa*, *B. qianbeiensis*, *B. shuichengensis*, *B. spinata*, and *B. wushanensis* (vs. toes with one-fourth in the latter).

By heels overlapping when thighs are positioned at right angles to the body, *Boulenophrys yezhongensis* sp. nov. differs from *B. acuta*, *B. brachykolos*, *B. daoji*, *B. dongguanensis*, *B. fengshunensis*, *B. gaolanensis*, *B. hengshanensis*, *B. hungtai*, *B. insularis*, *B. kuatunensis*, *B. lichun*, *B. nankunensis*, *B. obesa*, *B. ombrophila*, *B. pepe*, *B. puningensis*, *B. shuichengensis*, and *B. wugongensis* (vs. not meeting in the latter). differs from *B. binchuanensis*, *B. elongata*, *B. minor*, *B. xiangnanensis*, and *B. xuefengmontis* (vs. just meeting in the latter).

By tibiotarsal articulation reaching to the level to the middle of eye when leg stretched forward, *Boulenophrys yezhongensis* sp. nov. differs from *B. cheni*, *B. congjiangensis*, *B. daweimontis*, *B. lini*, *B. obesa*, *B. shunhuangensis and B. yangmingensis* (vs. reaching the anterior corner of the eye or beyond eye or nostril and tip of snout in the latter); and differs from *B. brachykolos*, *B. chishuiensis*, *B. daoji*, *B. dongguanensis*, *B. fengshunensis*, *B. hungtai*, *B. insularis*, *B. jiangi*, *B. kuatunensis*, *B. lishuiensis*, *B. mufumontana*, *B. nankunensis*, *B. puningensis*, *B. qianbeiensis*, *B. shimentaina*, *B. xiangnanensis*,

Figure 8. Habitats of *Boulenophrys yezhongensis* sp. nov. in the type locality, Yezhong Nature Reserve, Shuicheng County, Guizhou Province, China. **A.** Landscape of montane forests in the type locality; **B.** A mountain stream where *Boulenophrys yezhongensis* sp. nov. were encountered.

B. xuefengmontis, *B. yingdeensis and B. yunkaiensis* (vs. reaching the level between tympanum and eye or reach the tympanum or be even closer in the latter).

By having an internal single subgular vocal sac in males, *Boulenophrys yezhongensis* sp. nov. differs from *B. caudoprocta* and *B. shuichengensis* (vs. vocal sac absent in the latter).

Distribution and habitats. *Boulenophrys yezhongensis* sp. nov. is known from the type locality, Yezhong Nature Reserve, Shuicheng County, Guizhou Province, China, at elevations of approximately 2022 m. The individuals of the new species were frequently found on stones in the streams or gorges surrounded by evergreen broadleaved forest (Fig. 8), and two sympatric amphibian species, i.e., *Rana chaochiaoensis* and *Quasipaa boulengeri*, were found.

Etymology. The specific epithet *yezhongensis* refers to the type locality of the species. We propose the common English name "Yezhong Horned Toad" and the Chinese name "Yě Zhōng Jiǎo Chán (野钟角蟾)" for this species.

Discussion

Speciation in amphibians often occurs through a combination of spatial isolation and ecological specialization, particularly in montane regions where topographic complexity creates micro-refugia (Katharina et al. 2019; Schilthuizen 2000). Our integrative study demonstrates that *Boulenophrys yezhongensis* sp. nov. meets the criteria for species delimitation, despite its low 16S genetic divergence (0.6%) from *B. binlingensis*. While such minimal mitochondrial divergence might raise questions about species status, recent studies have shown that comparable or even lower interspecific genetic divergences have been recorded among anuran species with significant morphological and ecological differences in the genus *Zhangixalus* (e.g., the 16S genetic divergence between *Z. hui* and *Z. dugritei* is 0.58%; Pan et al. 2025). The congruence between discrete morphological characters (e.g., body size, webbing reduction, nuptial spine distribution) and bioclimatic niche partitioning strongly supports the recognition of this lineage as a distinct evolutionary unit under the framework of integrative taxonomy (Cheng et al. 2025).

The spatial isolation driving this speciation event is mediated by multiple geographic barriers. The new species is restricted to a single peak (2,022 m) within the Wumeng Mountain Range, separated from the nearest congeneric populations (*B. qianbeiensis* and *B. fanjingmontis*, at 1,400–1,800 m) by 12 km of intervening low-elevation valleys (<1,000 m) crossed by the deeply incised Beipan River (Fig. 1). This phylogeographic pattern mirrors speciation mechanisms observed in other East Asian montane frogs, where altitudinal stratification

and riverine barriers synergistically prevent gene flow (Li et al. 2015). The sharp elevational transition (>800 m vertical difference) between populations creates a thermal discontinuity equivalent to a 4–5 °C mean annual temperature differential – a critical threshold for amphibian physiological isolation (Wang et al. 2021; Jin et al. 2022). Furthermore, the karstic topography of the Yezhong region features extensive exposed limestone formations between populations, creating an ecological filter that could limit dispersal for these stream-dependent amphibians, as in other karst-endemic species (Wen et al. 2022).

This This microendemic species' confinement to a <0.5 km² stream system highlights the vulnerability of montane amphibians to environmental change. Its reproductive strategy – breeding in perennial cascades with stable low temperatures (14–16 °C) – suggests specialized adaptation to cold stenothermy, a trait increasingly recognized as both a speciation driver and extinction risk factor in tropical montane ecosystems (Kissel et al. 2018). Based on current surveys, *B. yezhongensis* sp. nov. is restricted to a limited area within the Yezhong Nature Reserve, with no populations detected in adjacent low-elevation regions. Following the IUCN Red List criteria (IUCN 2012) and considering its extremely small extent of occurrence (<20 km²) and single known location, we recommend classifying this species as Vulnerable (VU).

Institutional review board statement

All animal protocols in this study were approved by the Management Office of Yezhong Nature Reserve, Guizhou Province (project number: P52230020243100013). The Animal Care and Use Committee of Guizhou University provided full approval for the research protocol (approval number: EAE-GZU-20244-T1228).

Acknowledgments

This work was supported by the West Light Foundation of the Chinese Academy of Sciences (Grant No. 2021XBZG_XBQNXZ_A_006), the National Natural Science Foundation of China (Nos. 32270498, 31960099, 32260136, and 32070426), Guizhou Provincial Science and Technology Projects (Nos. ZK[2022]540 and [2023]099), and high-level personnel research start-up funding projects of the Moutai Institute (Nos. mygccrc[2022]055, mygccrc[2022]067, and mygccrc[2022]083).

References

Boulenger GA (1899) On a collection of reptiles and batrachians made by Mr. J. D. La Touche in N.W. Fokien, China. Proceedings of the Zoological Society of London 1899: 159–172. https://doi.org/10.1111/j.1469-7998.1899.tb06855.x

Bourret R (1937) Notes herpétologiques sur l'Indochine française. XIV. Les batraciens de la collection du Laboratoire des Sciences Naturelles de l'Université. Descriptions de quinze especes ou variétés nouvelles. Annexe au Bulletin Général de l'Instruction Publique Hanoi,1937: 5–56.

Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Molecular Biology and Evolution 17(4): 540–552. https://doi.org/10.1093/oxfordjournals.molbev.a026334

Chan KO, Hertwig ST, Neokleous DN, Flury JM, Brown RM (2022) Widely used, short 16S rRNA mitochondrial gene fragments yield poor and erratic results in phylogenetic estimation and species delimitation of amphibians. BMC Evolutionary Biology 22(1): 37. https://doi.org/10.1186/s12862-022-01994-y

Che J, Chen HM, Yang JX, Jin JQ, Jiang K, Yuan ZY, Murphy RW, Zhang YP (2012) Universal COI primers for DNA barcoding amphibians. Molecular Ecology Resource 12(2):247–258. https://doi.org/10.1111/j.1755-0998.2011.03090.x

Chen JM, Zhou WW, Nikolay A, Poyarkov Jr, Stuart BL, Brown RM, Lathrop A, Wang YY, Yuan ZL, Jiang K, Hou M, Chen HM, Suwannapoom C, Nguyen SN, Duong TV, Papenfuss TJ, Murphy RW, Zhang YP, Che J (2017) A novel multilocus phylogenetic estimation reveals unrecognized diversity in Asia toads, genus *Megophrys* sensu lato (Anura: Megophryidae). Molecular Phylogenetics and Evolution 106: 28–43. https://doi.org/10.1016/j.ympev.2016.09.004

Cheng R, Luo A, Orr M, Ge DY, Hou ZE, Qu YH, Guo BC, Zhang F, Sha ZL, Zhao Z, Wang MQ, Shi XY, Han HX, Zhou QS, Li YN, Liu XY, Shao C, Zhang AB, Zhou X, Zhu CD (2025) Cryptic diversity begets challenges and opportunities in biodiversity research. *Integrative Zoology* 20: 33–49. https://doi.org/10.1111/1749-4877.12809

Delorme M, Dubois A, Grosjean S, Ohler A (2006) Une nouvelle ergotaxinomie des Megophryidae (Amphibia, Anura). Alytes 24: 6–21.

Dubois A (1987) Miscellanea taxinomica batrachologica (I). Alytes 1987[1986]: 7–95.

Fei L, Hu SQ, Ye CY, Huang YZ (2009) Fauna Sinica. Amphibia (Vol. 2). Anura. Science Press, Beijing. [In Chinese]

Fei L, Ye CY, Huang YZ (1983) Two new subspecies of *Megophrys omeimontis* Liu from China (Amphibia, Pelobatidae). Acta Herpetologica Sinica. New Series, Chengdu, 2(2): 49–52. [In Chinese with English abstract]

Fei L, Ye CY, Huang YZ (1990) Key to Chinese Amphibians. Publishing House for Scientific and Technological, Chongqing, China. [In Chinese]

Fei L, Ye CY, Jiang JP (2012) Colored atlas of Chinese Amphibians and their distributions. Sichuan Publishing House of Science and Technology, Chengdu, China. [In Chinese]

Fei L, Ye CY, Jiang JP (2016) Genus *Liuophrys* Fei, Ye and Jiang, new genus; Subgenus *Atympanophrys* (*Borealophrys*) Fei, Ye and Jiang, new subgenus; Subgenus *Atympanophrys* (*Gigantophrys*) Fei, Ye and Jiang, new subgenus; Genus *Boulenophrys* Fei, Ye and Jiang, 2016, new genus; Subgenus *Xenophrys* (*Tianophrys*) Fei, Ye and Jiang, new subgenus. In: Fei L, Ye CY (Eds) Amphibians of China. Volume (I). Science Press, Beijing, China. 611–735.

Frost DR (2025) Amphibian Species of the World: an Online Reference. Version 6.0. Electronic Database. American Museum of Natural History, New York, USA. http://research.amnh.org/herpetology/amphibia/index.html [Accessed on: 2025-03-02]

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Systematic Biology 59(3): 07–321. https://doi.org/10.1093/sysbio/syq010

Hall TA (1999) BIOEDIT: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41(41): 95–98.

Herrel A, Gibbon R (2001) Use of isoflurane for euthanasia in amphibians. Journal of Herpetological Medicine and Surgery, 11(2): 68–71.

Hu SX, Zhao EM, Liu CZ (1973) A survey of amphibians and reptiles in Kweichow province, including a herpetofauna analysis. Acta Zoologica Sinica, 19(2): 149–171.

Inger RF, Romer JD (1961) A new pelobatid frog of the genus *Megophrys* from Hong Kong. Fieldiana. Zoology 39(46): 533–538. https://doi.org/10.5962/bhl.title.3373

IUCN (2012) IUCN Red List Categories and Criteria: Version 3.1. 2nd ed. Gland, Switzerland and Cambridge, UK: IUCN Species Survival Commission. https://www.iucnredlist.org/resources/categories-and-criteria

Jiang JP, Ye CY, Fei L (2008) A new horn toad *Megophrys sangzhiensis* from Hunan, China (Amphibia, Anura). Zoological Research 29(2): 219–222. [In Chinese with English abstract] https://doi.org/10.3724/SP.J.1141.2008.00219

Jin L, Zheng YC, Luan XF, Liao WB (2022) Genomic differentiation with isolation by distance along a latitudinal gradient in the spotted-leg treefrog *Polypedates megacephalus*. Integrative Zoology 18(5): 569–580. https://doi.org/10.1111/1749-4877.12654

Katharina C, Wollenberg V, Jonathon CM, Elizabeth B, Adalgisa C, Arley C, Mariana M, Matthew LN, Maciej P, Michael AR,Barry S, Fernanda PW, Jack WS, John JW, Sebastian SP (2019) Mechanisms and genetics of speciation in reptiles and amphibians. Genes 10(9): 646. https://doi.org/10.3390/genes10090646

Kissel AM, Palen W, Ryan ME, Adams MJ (2018) Compounding effects of climate change reduce population viability of a montane amphibian. Ecological Applications 29(2): e01832. https://doi.org/10.1002/eap.1832

Lathrop A (1997) Taxonomic review of the megophryid frogs (Anura: Pelobatoidea). Asian Herpetological Research 7: 68–79. https://doi.org/10.5962/bhl.part.18857

Lemmon AR, Moriarty EC (2004) The importance of proper model assumption in Bayesian phylogenetics. Systematic Biology 53(2): 265–277. https://doi.org/10.1080/10635150490423520

Li YL, Jin MJ, Zhao J, Liu ZY, Wang YY, Pang H (2014) Description of two new species of the genus *Megophrys* (Amphibia: Anura: Megophryidae) from Heishiding Natural Resreve, Fengkai, Guangdong, China, based on molecular and morphological data. Zootaxa 3795(4): 449–471. https://doi.org/10.11646/zootaxa.3795.4.5

Li SZ, Xu N, Liu J, Jiang JP, Wei G, Wang B (2018) A New Species of the Asian Toad Genus *Megophrys sensu lato* (Amphibia: Anura: Megophryidae) from Guizhou Province, China. Asian Herpetological Research 9(4): 224–239. https://doi.org/10.16373/j.cnki.ahr.180072

Li SZ, Lu NN, Liu J, Wang B (2020) Description of a new *Megophrys* Kuhl & Van Hasselt, 1822 (Anura, Megophryidae) from Guizhou Province, China. ZooKeys 986: 101–126. https://doi.org/10.3897/zookeys.986.57119

Li YM, Wu XY, Zhang HB, Yan P, Xue H, Wu XB (2015) Vicariance and its impact on the molecular ecology of a Chinese ranid frog species-complex (*Odorrana schmackeri*, Ranidae)." PLoS One 10: e0138757. https://doi.org/10.1371/journal.pone.0138757

Lin SS, Chen HH, Li YH, Peng ZN, Zeng ZC, Wang J (2024) A new *Boulenophrys* species (Anura, Megophryidae) from the coastal hills of eastern Fujian Province, China. ZooKeys 1216: 1–15. https://doi.org/10.3897/zookeys.1216.130017

Liu CZ (1950) Amphibians of Western China. Fieldiana. Zoology Memoires 2: 1–400. https://doi.org/10.5962/bhl.part.4737

Liu ZY, Zhu TQ, Zeng ZC, Lyu ZT, Wang J, Messenger K, Greenberg A J, Gou ZX, Yang ZH, Shi SH, Wang YY (2018) Prevalence of cryptic species in morphologically uniform taxa–Fast speciation and evolutionary radiation in Asian frogs. Molecular Phylogenetics and Evolution 127: 723–731. https://doi.org/10.1016/j.ympev.2018.06.020

Liu J, Li SZ, Wei G, Xu N, Cheng YL, Wang B, Wu J (2020) A new species of the Asian toad genus *Megophrys sensu lato* (Anura: Megophryidae) from Guizhou Province, China. Asian Herpetological Research 11(1): 1–18. https://doi.org/10.16373/j.cnki.ahr.190041

Lleonart J, Salat J, Torres GJ (2000) Removing allometric effects of body size in morphological analysis. Journal of Theoretical Biology 205: 85–93. https://doi.org/10.1006/jtbi.2000.2043

Luo T, Wang Y, Wang S, Lu X, Wang W, Deng H, Zhou J (2021) A species of the genus *Panophrys* (Anura, Megophryidae) from southeastern Guizhou Province, China. ZooKeys 1047: 27–60. https://doi.org/10.3897/zookeys.1047.61097

Lyu Z-T, Huang L-S, Wang J, Li Y-Q, Chen H-H, Qi S, Wang Y-Y (2019) Description of two cryptic species of the *Amolops ricketti* group (Anura, Ranidae) from southeastern China. ZooKeys 812: 133–156. https://doi.org/10.3897/zookeys.812.29956

Lyu Z-T, Li Y-Q, Zeng Z-C, Zhao J, Liu Z-Y, Guo G-X, Wang Y-Y (2020) Four new species of Asian horned toads (Anura, Megophryidae, *Megophrys*) from southern China. ZooKeys 942: 105–140. https://doi.org/10.3897/zookeys.942.47983

Lyu Z-T, Qi S, Wang J, Zhang S-Y, Zhao J, Zeng Z-C, Wan H, Yang J-H, Mo Y-M, Wang Y-Y (2023) Generic classification of Asian Horned Toads (Anura: Megophryidae: Megophryinae) and monograph of Chinese species. Zoological Research. Kunming 44: 380–450. https://doi.org/10.24272/j.issn.2095-8137.2022.372

Lyu Z-T, Zeng Z-C, Wang J, Liu Z-Y, Huang Y-Q, Li W-Z, Wang Y-Y (2021) Four new species of *Panophrys* (Anura, Megophryidae) from eastern China, with discussion on the recognition of *Panophrys* as a distinct genus. Zootaxa 4927(1):9–40. https://doi.org/10.11646/zootaxa.4927.1.2

Mahony S, Nicole MF, Biju SD, Teeling EC (2017) Evolutionary history of the Asian Horned Frogs (Megophryinae): integrative approaches to timetree dating in the absence of a fossil record. Molecular Phylogenetics and Evolution 34(3): 744–771. https://doi.org/10.1093/molbev/msw267

McGuire JA, Witt CC, Altshuler DL, Remsen JV (2007) Phylogenetic systematics and biogeography of hummingbirds: Bayesian and maximum likelihood analyses of partitioned data and selection of an appropriate partitioning strategy. Systematic Biology 56(5): 837–856. https://doi.org/10.1080/10635150701656360

Messenger KR, Dahn HA, Liang YR, Xie P, Wang Y, Lu CH (2019) A new species of the genus *Megophrys* Gunther, 1864 (Amphibia: Anura: Megophryidae) from Mount Wuyi, China. Zootaxa 4554(2): 561–583. https://doi.org/10.11646/zootaxa.4554.2.9

Mo XY, Shen YH, Li HH, Wu MS (2010) A new species of *Megophrys* (Amphibia: Anura: Megophryidae) from the northwestern Hunan Province, China. Current Zoology 56(4): 432–436. https://doi.org/10.1093/czoolo/56.4.432

Nguyen TQ, Pham CT, Nguyen TT, Luong AM, Ziegler T (2020) A new species of *Megophrys* (Amphibia: Anura: Megophryidae) from Vietnam. Zootaxa 4722: 401–422. https://doi.org/10.11646/zootaxa.4722.5.1

Pan YQ, Liu S, Chen J, Yu GH (2025) Underestimated species diversity in *Zhangixalus* (Anura, Rhacophoridae) with a description of two cryptic species from southern China. Zoosystematics and Evolution 101(2): 485–507. https://doi.org/10.3897/zse.101.144060

Pope CH (1929) Four new frogs from Fukien Province, China. American Museum Novitates 352: 1–5. http://digitallibrary.amnh.org/handle/2246/4057

Qi S, Lyu ZT, Wang J, Mo YM, Zeng ZC, Zeng YJ, Dai KY, Li YQ, Grismer LL, Wang YY (2021) Three new species of the genus *Boulenophrys* (Anura, Megophryidae) from southern China. Zootaxa 5072(5): 401–438. https://doi.org/10.11646/zootaxa.5072.5.1

Qian TY, Hu K, Mo XY, Gao Z, Zhang N, Yang DD (2023) A new species of *Boulenophrys* from central Hunan Province, China (Anura: Megophryidae). Vertebrate Zoology 73: 915–930. https://doi.org/10.3897/vz.73.e100889

Rao DQ, Yang DT (1997) The karyotypes of Megophryinae (Pelobatidae) with a discussion on their classification and phylogenetic relationships. Asian Herpetological Research 7: 93–102. https://doi.org/10.5962/bhl.part.18858

Robert L, Brett C, Simon YWH, Stephane G (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Molecular Phylogenetics and Evolution 29(6): 1695–1701. https://doi.org/10.1093/molbev/mss020

Ronquist FR, Huelsenbeck JP (2003) MrBayes3: Bayesian phylogenetic inference under mixedmodels. Bioinformatics 19(12): 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York, America.

Schilthuizen M (2000) Dualism and conflicts in understanding speciation. BioEssays 22(12): 1134–1141. https://doi.org/10.1002/1521-1878(200012)22:12<1134::AIDBIES11>3.0.CO;2-5

Shen YH (1994) A new pelobatid toad of the genus *Megophrys* from China (Anura: Pelobatidae). In: Zoological Society of China Editor. The 60th Anniversary of the Foundation of the Zoological Society of China, Nanking (China), September 1994. China Science and Technology Publishing House, 603–606.

Shi S, Li D, Zhu W, Jiang W, Jiang J, Wang B (2021) Description of a new toad of *Megophrys* Kuhl & Van Hasselt, 1822 (Amphibia: Anura: Megophryidae) from western Yunnan Province, China. Zootaxa 4942(3): 351–381. https://doi.org/10.11646/zootaxa.4942.3.3

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society of America 87(6): 651–701. https://doi.org/10.1093/aesa/87.6.651

Song HM, Wang HT, Qi S, Wang N, Wang YY (2024) A new species of the genus *Boulenophrys* (Anura: Megophryidae: Megophryinae) from Zhuhai, Guangdong, China. Asian Herpetological Research 15(4): 251–264. https://doi.org/10.3724/ahr.2095-0357.2024.0026

Stejneger L (1926) Two new tailless amphibians from western China. Proceedings of the Biological Society of Washington 39: 53–54.

Su HJ, Shi SC, Wu YQ, Li GR, Yao XG, Wang B, Li SZ (2020) Description of a new horned toad of *Megophrys* Kuhl & Van Hasselt, 1822 (Anura, Megophryidae) from southwest China. ZooKeys 974: 131–159. https://doi.org/10.3897/zookeys.974.56070

Tamura K, Stecher G, Peterson D, Fiipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis, version 6.0. Molecular Biology and Evolution 30: 2725–2729. https://doi.org/10.1093/molbev/mst197

Tapley B, Cutajar T, Mahony S, Nguyen CT, Dau VQ, Nguyen TT, Luong HV, Rowley JJL (2017) The Vietnamese population of *Megophrys kuatunensis* (Amphibia: Megophryidae) represents a new species of Asian horned frog from Vietnam and southern China. Zootaxa 4344(3): 465–492. https://doi.org/10.11646/zootaxa.4344.3.3

Tapley B, Cutajar TP, Mahony S, Nguyen CT, Dau VQ, Luong AM, Le DT, Nguyen TT, Nguyen TQ, Portway C, Luong HV, Rowley JJL (2018) Two new and potentially highly threatened *Megophrys* Horned frogs (Amphibia: Megophryidae) from Indochina's highest mountains. Zootaxa 4508: 301–333. https://doi.org/10.11646/zootaxa.4508.3.1

Tapley B, Cutajar TP, Nguyen LT, Portway C, Mahony S, Nguyen CT, Harding L, Luong HV, Rowley JJL (2021) A new potentially Endangered species of *Megophrys* (Amphibia: Megophryidae) from Mount Ky Quan San, north-west Vietnam. Journal of Natural History, 54: 2543–2575. https://doi.org/10.1080/00222933.2020.1856952

Tian YZ, Sun A (1995) A new species of *Megophrys* from China (Amphibia: Pelobatidae). Journal of Liupanshui Teachers College 52(3): 11–15. [In Chinese]

Wang B, Wu YQ, Peng JW, Shi SC, Lu NN, Wu J (2020) A new *Megophrys* Kuhl and Van Hasselt (Amphibia: Megophryidae) from southeastern China. ZooKeys 904: 35–62. https://doi.org/10.3897/zookeys.904.47354

Wang J, Liu ZY, Lyu ZT, Wang YY (2017a) A new species of the genus *Megophrys* (Amphibia: Anura: Megophryidae) from an offshore island in Guangdong Province, southeastern China. Zootaxa 4324(3): 541–556. https://doi.org/10.11646/zootaxa.4324.3.8

Wang YE, Liu BQ, Jiang K, Jin W, Xu JN, Wu CH (2017b) A new species of the Horn Toad of the genus *Xenophrys* from Zhejiang, China (Amphibia: Megophryidae). Chinese Journal of Zoology 52: 19–29. [in Chinese with English abstract]

Wang YY, Zhang TD, Zhao J, Sung YH, Yang JH, Pang H, Zhang Z (2012) Description of a new species of the genus *Megophrys* Günther, 1864 (Amphibia: Anura: Megophryidae) from Mount Jinggang, China, based on molecular and morphological data. Zootaxa 3546: 53–67. https://doi.org/10.11646/zootaxa.3546.1.4

Wang YY, Zhao J, Yang JH, Zhou ZM, Chen GL, Liu Y (2014) Morphology, molecular genetics, and bioacoustics support two new sympatric *Megophrys* (Amphibia: Anura Megophryidae) species in Southeast China. PLoS ONE 9: e93075: 1–15. https://doi.org/10.1371/journal.pone.0093075

Wang L, Deng XJ, Liu Y, Wu QQ, Liu Z (2019a) A new species of the genus *Megophrys* (Amphibia: Anura: Megophryidae) from Hunan, China. Zootaxa 4695 (4): 301–330. https://doi.org/10.11646/zootaxa.4695.4.1

Wang J, Lyu ZT, Liu ZY, Liao CK, Zeng ZC, Li YL, Wang YY (2019b) Description of six new species of the subgenus *Panophrys* within the genus *Megophrys* (Anura, Megophryidae) from southeastern

China based on molecular and morphological data. ZooKeys 851: 113–164. https://doi.org/10.3897/zookeys.851.29107

Wang J, Zeng ZC, Lyu ZT, Qi S, Liu ZY, Chen HH, Lu YH, Xiao HW, Lin CR, Chen K, Wang YY (2022) Description of three new *Boulenophrys* species from eastern Guangdong, China, emphasizing the urgency of ecological conservation in this region (Anura, Megophryidae). Zootaxa 5099: 91–119. https://doi.org/10.11646/zootaxa.5099.1.4

Wang J, Lin SS, Gan JS, Chen HH, Yu LM, Pan Z, Xiao JJ, Zeng ZC (2024) A new species of the genus *Boulenophrys* from South China (Anura, Megophryidae). Zootaxa 5514(5): 451–468. https://doi.org/10.11646/zootaxa.5514.5.3

Wang XY, Zhong MJ, Zhang J, Si XF, Yang SN, Jiang JP, Hu JH (2021) Multidimensional amphibian diversity and community structure along a 2600 m elevational gradient on the eastern margin of the Qinghai-Tibetan Plateau. Zoological Research 43(1): 40–51.

Wen HM, Luo T, Wang YL, Wang SW, Liu T, Xiao N, Zhou J (2022)Molecular phylogeny and historical biogeography of the cave fish genus *Sinocyclocheilus* (Cypriniformes: Cyprinidae) in Southwest China. Integrative Zoology 17(2): 311–325. https://doi.org/10.1111/1749-4877.12624

Wheeler WC (2016) Phylogenetic analysis: Concepts and practice (3rd ed.). John Wiley & Sons.

Wu Y, Li S, Liu W, Wang B, Wu J (2020) Description of a new horned toad of *Megophrys* Kuhl & Van Hasselt, 1822 (Amphibia, Megophryidae) from Zhejiang Province, China. ZooKeys 1005: 73–102. https://doi.org/10.3897/zookeys.1005.58629

Wu YH, Suwannapoom C, Poyarkov Jr NA, Chen JM, Pawangkhanant P, Xu K, Jin JQ, Murphy RW, Che J (2019) A new species of the genus *Xenophrys* (Anura: Megophryidae) from northern Thailand. Zoological Research 40: 564–574. https://doi.org/10.24272/j.issn.2095-8137.2019.032

Xiao B, Xi J, Shi S, Li H, Zhu L, Maimaiti A, Xu Y, Liao S, Wang B, Mo X (2025) A new species of the genus *Boulenophrys* (Anura, Megophryidae) from Southern Hunan Province, Central China. Animals 15: 440. https://doi.org/10.3390/ani15030440

Xu N, Li SZ, Liu J, Wei G, Wang B (2020) A new species of the horned toad *Megophrys* Kuhl & Van Hasselt, 1822 (Anura, Megophryidae) from southwest China. ZooKeys 943: 119–144. https://doi.org/10.3897/zookeys.943.50343

Ye CY, Fei L (1995) Taxonomic studies on the small type *Megophrys* in China including descriptions of the new species (subspecies) (Pelobatidae: genus *Megophrys*). Herpetologica Sinica 4–5: 72–81. [In Chinese]

Ye CY, Fei L, Xie F (2007) A new species of Megophryidae *Megophrys baolongensis* from China (Amphibia, Anura). Herpetologica Sinica 11: 38–41. [In Chinese]

Zeng ZC, Wang J, Chen HH, Xiao WW, Zhan BB, Li YH, Lin SS (2024) A new species of the genus *Boulenophrys* (Anura, Megophryidae) from eastern Guangdong, China. Asian Herpetological Research 15(1): 12–21. https://doi.org/10.3724/ahr.2095-0357.2023.0038

Zhang L, Liang L, Ran H, Shen ZX (2012) *Megophrys binlingensis fanjingmontis*: a new subspecies of Megophryidae from Guizhou, China. Chinese Journal Zoology 47: 135–138. [In Chinese]

Zhang Y, Li G, Xiao N, Li J, Pan T, Wang H, Zhang B, Zhou J (2017) A new species of the genus *Megophrys* (Amphibia: Anura: Megophryicae) from Libo County, Guizhou, China. Asian Herpetological Research 8: 75–85. https://doi.org/10.16373/j.cnki.ahr.160041

Supplementary material 1

Morphometric data used for morphological characteristic analysis

Authors: Jing Liu, Chao-Bo Feng,Tuo Shen, Shi-Ze Li, Yanlin Cheng, Gang Wei, Bin Wang, Haijun Su

Data type: xlsx

Explanation note: Measurements of the adult specimens of *Boulenophrys yezhongensis* sp. nov. Units are in mm. See abbreviations for the morphological characters in Materials and methods section.

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/herpetozoa.38.e155430.suppl1

Supplementary material 2

Genetic distance of 16S sequences

Authors: Jing Liu, Chao-Bo Feng,Tuo Shen, Shi-Ze Li, Yanlin Cheng, Gang Wei, Bin Wang, Haijun Su

Data type: xls

Explanation note: Uncorrected *p*-distances between the *Boulenophrys* species based on the 16S gene sequences.

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/herpetozoa.38.e155430.suppl2

Supplementary material 3

Genetic distance of COI sequences

Authors: Jing Liu, Chao-Bo Feng,Tuo Shen, Shi-Ze Li, Yanlin Cheng, Gang Wei, Bin Wang, Haijun Su

Data type: xls

Explanation note: Uncorrected *p*-distances between the *Boulenophrys* species based on the COI gene sequences.

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/herpetozoa.38.e155430.suppl3

Supplementary material 4

Diagnostic characters separating *Boulenophrys yezhongensis* sp. nov. from other species of *Boulenophrys*

Authors: Jing Liu, Chao-Bo Feng,Tuo Shen, Shi-Ze Li, Yanlin Cheng, Gang Wei, Bin Wang, Haijun Su

Data type: xlsx

Explanation note: Morphological characteristic differences in *Boulenophrys* are used for identification.

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/herpetozoa.38.e155430.suppl4